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NO. I.

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A SALT SOLUTION IN LOCOMOTOR ATAXIA.

BY SAMUEL A. MATTHEWS AND ORVILLE H. BROWN.

*[From the Hull Physiological Laboratory of the University of Chicago.]*

IN the pathological condition known as locomotor ataxia or tabes dorsalis, the anatomical changes are fairly well understood, but the physiology of the disease, notwithstanding the fact that it has been the subject of much study, is as yet very incompletely known. The chief symptoms result from nerve impairment, which may be evidenced by an increase or decrease of sensation in any part of the body. It is usually in the lower limbs, thus causing difficult walking or standing, and around the waist, causing the girdle sensation. During the early stages of the disease the affected parts show irregular areas of both hyperæsthesia and partial anæsthesia. As the disease progresses, the areas and the degree of anæsthesia increase. It is not within the scope of this paper to discuss the pathology of locomotor ataxia. Suffice it to say that the anatomical changes in the cord and in the peripheral processes of the spinal ganglia cells in advanced stages of the disease are of such a nature as to preclude any restoration of function.

The treatment of the malady usually recommended is purely hygienic, and its results are unsatisfactory. It is well known that the condition of such patients apparently fluctuates, and that almost any treatment causes them to believe that an improvement has been effected. However, under favorable conditions, the course of the disease may be arrested, and occasionally an actual improvement may occur.

Under very peculiar circumstances, about a year ago, one case of tabes dorsalis was very hesitatingly and cautiously treated by an injection of the following solution of salts: 250 c.c.  $\frac{m}{8}$  sodium chloride, 125 c.c.  $\frac{m}{8}$  sodium sulphate, 120 c.c.  $\frac{m}{12}$  sodium citrate, and 5 c.c.  $\frac{m}{8}$  calcium chloride. Since that time nine patients suffering from the same malady have been similarly treated. The results, though quite void of therapeutic value, are interesting from a physiological standpoint.

*Case I, February 1, 1903.* — Mr. P——, age 56, medium height; poorly nourished. The typical symptoms of locomotor ataxia were present. According to the patient's statement, the affliction had been well defined for fifteen years. The anæsthesia was almost complete, and included the feet, ankles, legs, extending upwards to a line about one and a half inches above the condyles of the femur, and an area along the small of the back. The man had formerly suffered extremely from the pains which are so characteristic of the disease. These pains were always confined to the areas which at the time of treatment were anæsthetic. For the past five years the pains had been only occasional, and were not of great severity. The solution injected is mentioned above. Between 3 and 5 P.M. 400 c.c. of the mixture were injected into the tissues of the upper lumbar region. At this time no uncomfortable feeling was observed by the patient. He noticed that his feet were warm, which was unusual. About 8.30 in the evening, severe pains began in the legs. Concurrent with the beginning of the pains, was a general nervous shock, characterized by a muscular tremor which was particularly evidenced in the inferior maxilla, and which produced a chattering of the teeth similar to that of a chill. However, the patient was not cold, nor was there any rise of temperature observed by the patient. The general nervousness lasted for only a few hours, but the pains continued for about forty-eight hours, and then gradually subsided. These pains were confined practically to those areas which the physical examination showed to be anæsthetic, and, according to the statement of the patient, the pains were not only substantially the same in character and intensity as those of previous years, but were confined to the same areas, and began in the area first affected by the disease.

*February 15.* — A mixture of 250 c.c.  $\frac{m}{8}$  sodium chloride, 75 c.c.  $\frac{m}{8}$  sodium sulphate, 75 c.c.  $\frac{m}{8}$  sodium citrate, 5 c.c.  $\frac{m}{8}$  calcium chloride, and 95 c.c. distilled water, was injected as on February 1. No uncomfortable effects were apparent until four and a half hours after the injection, when the patient was again attacked by the lightning-like pains which had followed the injection on February 1. But the intensity of these pains was very much less, and they lasted only about fifteen hours.

*February 22.* — The patient was similarly treated as on the 15th. The pains began about four or five hours after the injection, and lasted for about twelve hours, being confined to the same areas. These pains were very much weaker than those occurring after the treatment on the 15th.

*March 14.* — The solution used in the first treatment was injected. There was little or no reaction.

*Case II, March 3, 1903.* — Mr. Mc——, age 53, a large, well-nourished man. About five or six years ago symptoms developed which were characteristic of locomotor ataxia. The case was now well defined. Sensation was impaired in the lower limbs, especially below the knees. The pains which had troubled him for several years, and from which he still suffered, were confined to the lower limbs, and were more marked in the places where the anæsthesia was the greatest. He also had abdominal pains (the so-called visceral crises). The same salts used in Case I were similarly used in this case.

In general, the effect of the injections was the same as in Case I, *i. e.*, the characteristic ataxia pains were produced in the areas affected. There was a recurrence of gastro-intestinal disturbance, which consisted of severe pains and vomiting. These symptoms followed each injection, with lessened intensity, until about the fifth treatment, after which they were almost or entirely absent. Following the injection, and lasting for ten or fifteen hours, there was an increase of fifteen or twenty beats in the rate of the pulse, and a rise of about 2° F. in temperature. An injection was made each week, until eight had been given. The last three caused little or no pain, but the same pulse and temperature reactions were still present; there was also a nervousness which prevented sleep for about fifteen hours following the injections.

*Case III.* — Mr. N——, age 35. Printer by trade. For the past four years his eyes had been troubling him, and he finally had to give up his vocation. His case had not been diagnosed as locomotor ataxia until about January 1 of the present year. He had noticed for some time a numbness of the little and ring fingers, and of the corresponding portion of the hand and forearm of the left arm. No pains had been felt in arms, face, or abdomen, but he had been troubled with what he called "rheumatic pains and a general ache" below the knees. On rising, his legs were stiff and slightly swelled. The physical examination showed that his sensation to touch was everywhere very good, except in his feet and ankles to about three inches above the prominence of the ankle joints. On being touched with cotton, a small area of each instep was found to be hypersensitive, otherwise sensation below the line above-mentioned was either absent or very slight.

On January 31, 400 c.c. of a saline solution similar to that previously mentioned, was injected into the tissues of the upper lumbar region a

little to the left of the median line. Within about three hours after the conclusion of the injection uncomfortable sensations began, which were characterized by a peculiar diffused ache in the lower limbs, especially below the knees. He also experienced a very peculiar sensation, which he said was hardly a pain, in the area of the left forearm and hand. These peculiar sensations, bordering on pain, did not entirely leave him for about forty-eight hours.

The other cases corroborate the results given here. The salt solution has been injected into persons who did not have locomotor ataxia, but without producing similar sensations.

#### SUMMARY AND CONCLUSIONS.

1. The injection of a mixture of 250 c.c.  $\frac{m}{8}$  sodium chloride, 125 c.c.  $\frac{m}{8}$  sodium sulphate, 120 c.c.  $\frac{m}{12}$  sodium citrate, and 5 c.c.  $\frac{m}{8}$  calcium chloride, into the subcutaneous tissues of a man suffering from locomotor ataxia, causes pains and other sensations similar to those experienced in the earlier stages of the disease. This occurs even though it has been years since such pains were first felt. The pains decrease successively after each injection, until about the fourth or fifth, after which they may not occur at all.

2. We cannot state that such injections have any therapeutic value, though their effects are of physiological interest.

3. It is possible that the remarkable localization reaction may be of diagnostic value.

Our thanks are due to Professor Stewart and Dr. A. P. Mathews for criticisms, and to Mr. E. T. Hanley of the class of 1905 of Rush Medical College for his assistance in a number of the treatments.

## THE PATHS OF EXCRETION FOR INORGANIC COMPOUNDS. I.—THE EXCRETION OF STRONTIUM.

By LAFAYETTE B. MENDEL AND HENRY CLARKE THACHER.

*[From the Sheffield Laboratory of Physiological Chemistry, Yale University.]*

### I.

THE long-established custom of regarding the kidney as the only organ of importance involved in the elimination of either foreign or naturally occurring inorganic compounds from the body in the case of the higher animals, has repeatedly led physiologists into error. Various older theories of the absorption of iron derivatives afford a conspicuous illustration of this. The failure to recognize the intestinal epithelium as a factor concerned in the removal of iron from the system naturally allowed a false interpretation to be placed upon the occurrence of this element in the fæces. To the earlier observers, iron in the stools was a direct indication of lack of iron-absorption, particularly in view of the extremely scanty elimination of ferric salts usually noted in the urine during the same period. But the establishment of the fact that the gut may be directly concerned in the excretion as well as in the absorption of the iron compounds made possible a new interpretation of the earlier observations. Iron might now be present in the contents of the alimentary canal, either owing to the failure to be absorbed, or equally well because it had been discharged into this channel through the epithelial walls. It became necessary to follow the paths of elimination when the introduction of the element directly into the alimentary canal was avoided. And since it has been found that iron may be introduced either subcutaneously or even directly into the blood-current, without producing any marked increase in the output in the urine, while the fæces may contain noticeable quantities of it, the significance of the intestine with reference to the elimination of this element becomes apparent.

A more careful study of the excretory channels for other elements has revealed somewhat similar conditions, particularly in the case

calcium<sup>1</sup> and of phosphorus compounds.<sup>2</sup> Various organic poisons, including morphine and snake venoms, have long been known to be secreted into the stomach and other parts of the alimentary canal when they are introduced subcutaneously into the body.<sup>3</sup> Among inorganic substances, the salts of manganese appear to show a behavior entirely comparable with that of iron; magnesium, bismuth, zinc, lithium, and caesium also enter the intestine in noticeable quantities when they are introduced in ways other than per os; while under similar conditions nickel, cobalt, lead, arsenic, and mercury have been detected in the faeces.<sup>4</sup> In most cases, however, the quantitative data available are too meagre to afford any convincing conclusion regarding the relative importance of the gut as an excretory channel for these elements. Observations like those recently reported by Good, in which lithium was found to be excreted into the stomach and intestine in larger quantity than through the kidneys, indicate the desirability of more extensive investigation carried out with this point in mind. Not only are we for the most part uninstructed regarding the relative participation of the gut as an excretory organ under conditions like those, for example, which pertain in renal insufficiency; but we are also just beginning to appreciate the significance of such an excretory function in the local reaction and responses throughout the intestine, and the consequent therapeutic possibilities. Thus the treatment of certain intoxications by gastric lavage, and the more rational interpretation of the mode of action of the saline purgatives

<sup>1</sup> Cf. REY: *Archiv für experimentelle Pathologie und Pharmakologie*, 1895, xxxv, p. 295.

<sup>2</sup> Cf. NOËL PATON, DUNLOP and AITCHISON: *Journal of physiology*, 1900, xxv, p. 212; BERGMANN: *Archiv für experimentelle Pathologie und Pharmakologie*, 1901, xlvii, p. 77; also unpublished experiments by Dr. F. P. UNDERHILL in this laboratory.

<sup>3</sup> Cf. KUNKEL: *Handbuch der Toxikologie*, 1901, pp. 54-55.

<sup>4</sup> Cf. HARNACK: *Archiv für experimentelle Pathologie und Pharmakologie*, 1901, xlv, p. 372 (manganese); MEYER und STEINFELD: *Ibid.*, 1885, xx, p. 40 (bismuth); LUSK: *American text-book of physiology* (magnesium); SACHER: *Arbeiten aus dem pharmakologischen Institut, Dorpat*, 1893, ix, p. 88 (zinc); GOOD: *American journal of the medical sciences*, 1903, cxxv, p. 273 (lithium); HANFORD: *This journal*, 1903, ix, p. 214 (caesium); STUART: *Journal of anatomy and physiology*, 1884, xviii, p. 89 (nickel and cobalt); MANN: *British medical journal*, 1893, Feb. 25 (lead); HEFFTER: *Ergebnisse der Physiologie*, 1903, ii, (1), pp. 116-117 (arsenic); SCHUSTER: *Jahresbericht für Thierchemie*, 1882, xii, p. 118 (mercury). The preceding list of references is not intended in any way to be exhaustive.



in the light of recent work are at once suggested.<sup>1</sup> With considerations of this sort in view the writer has for some time had in progress a series of observations planned to extend our knowledge of the paths of excretion of various substances.<sup>2</sup> The experimental work recorded in this paper on strontium-excretion has been undertaken almost entirely by Mr. Thacher.

## II.

The salts of strontium have been selected for study in the present instance because of its chemical relationship to calcium, — an element involved in significant physiological reactions, — rather than in view of any demonstrated therapeutic properties. Furthermore, since it is never found normally in animals, and is readily detected in small quantities by its spectroscopic properties, strontium may readily be traced in the organism. Its compounds are usually stated to resemble those of barium and calcium in their general physiological and toxicological action. Considerable doses have been given by way of the mouth without producing serious symptoms. There are, however, almost no data at hand regarding the extent of its absorption and the paths of elimination.<sup>3</sup> Strontium does not appear to induce chronic forms of intoxication, although it has been found stored up in the bones of growing animals, possibly replacing calcium under these conditions. After feeding dogs for many days with strontium salts, Laborde<sup>4</sup> found traces of the metal in the urine, while the *faeces* contained it in larger amounts. From the bones of a dog which had received strontium phosphate equivalent to 265 gms. of the metal in the course of 111 days, only 0.63 gm. could be recovered. The observations suggest that strontium may leave the body in the urine, while they give little indication regarding the extent of actual absorption. The study of the distribution of strontium bromide carried out by Féré<sup>5</sup> and others offers no direct evidence. The experiments made on dogs by Raudnitz<sup>6</sup> to determine the place and conditions for the

<sup>1</sup> Cf. MACCALLUM: This journal, 1903, x, p. 101 and p. 259.

<sup>2</sup> Observations on the elimination of *cæsium* have already been published from this laboratory by G. A. HANFORD in this journal, 1903, ix, p. 235.

<sup>3</sup> Cf. HEFFTER: *Ergebnisse der Physiologie*, 1903, ii, (1), p. 121.

<sup>4</sup> LABORDE: *Comptes rendus de la société de biologie*, 1890, xlii, p. 453 and p. 458; 1891, xliii, p. 562.

<sup>5</sup> FÉRÉ: *Ibid.*, 1892, xliv, p. 513.

<sup>6</sup> RAUDNITZ: *Archiv für experimentelle Pathologie und Pharmakologie*, 1893, xxxi, p. 343.

absorption of the alkali earth (calcium and strontium) salts showed that this process goes on largely in the duodenum, the soluble chlorides being absorbed far more rapidly than the phosphates and carbonates. This investigator was unable to detect strontium in the ligated ileum, cæcum or large intestine, although traces were found in the liver and in the urine. In one case in which 0.62 gm. of strontium chloride was introduced into the jugular vein, the element could not be detected in the intestinal contents five hours later, although it had disappeared completely from the blood. In endeavoring to obtain some proof of the absorption of strontium, H. C. Wood, Jr.,<sup>1</sup> confirmed on man the observations of Laborde. He found traces of the metal in the urine of the first day, but none on the second day following the administration of 3 gms. of the lactate (1.89 gms. strontium) per os. During the same time 10 per cent of the strontium ingested was recovered from the fæces. Wood writes: "This result is capable of several explanations; it is possible that the strontium is not absorbed and very slowly passes out from the alimentary canal, owing to its weight causing it to cling to the mucous membranes; or it is possible that being more largely absorbed than is apparent, it is eliminated with great slowness. The latter conclusion, however, is highly improbable in the face of the fact that the strontium disappeared from the urine within twenty-four hours. If absorption and retention were the case, certainly there should have been as much strontium in the urine on the second as on the first day. It is, of course, possible that the discharge of strontium with the fæces may have depended upon an elimination of absorbed strontium by the intestinal mucous membrane." In an experiment directed toward the determination of the latter possibility, a dog was given strontium lactate hypodermically, but no strontium was detected within seventy-two hours in either urine or fæces; on the other hand, H. C. Wood, Sr. and Arnold<sup>2</sup> recovered 0.2 gm. strontium sulphate from the fæces of a dog which had received 2 gms. strontium nitrate hypodermically, while the total urine of the subsequent ten days contained only a minimal quantity. The same investigators, experimenting on man, found that soluble salts of strontium taken by way of the mouth do not escape from the body in the urine to any extent.

The few investigations just reviewed leave much to be ascertained regarding the fate of strontium in the body. They merely indicate that its salts may be absorbed in part at least and eliminated in

<sup>1</sup> H. C. WOOD, JR.: This journal, 1898, i, p. 83.

<sup>2</sup> H. C. WOOD and J. P. ARNOLD: Philadelphia medical journal, April, 1899.

traces in the urine; and unless there has been long-continued administration, the strontium is reported to disappear from the latter within a very short time. No light is thrown upon other possible channels for the ultimate removal of the foreign salt, further than in the experiments already quoted.

### III.

The new observations to be recorded were made upon dogs, cats, and a rabbit. Since the experiments were designed especially to determine the function of the gastro-intestinal canal in excretion, it became desirable to introduce the strontium in some way other than *per os*. Sterile solutions containing about four per cent of strontium acetate were introduced subcutaneously with aseptic precautions, and their distribution was facilitated by gentle massage; intraperitoneal and intravenous injections were also tried. After the injections, the skin was always carefully cleansed to avoid the possibility of having traces of strontium licked off by the animals. The latter were kept in metallic cages, and the urine and *fæces* were separately collected, unless this was made impossible by diarrhœa (which added considerable difficulty to our earlier experiments). A mixed diet was found to prevent these disturbances to a considerable extent.

**Methods of analysis.** — In the examination of the *fæces* and tissues for strontium, a slight modification of the method used by H. C. Wood, Jr.,<sup>1</sup> was followed. The dried materials were incinerated with the aid of ammonium nitrate, and then fused in a nickel or silver dish with potassium hydrate and sodium carbonate. The fusion mass containing the insoluble carbonates of strontium and calcium, etc., was extracted with hot water, the residue dissolved in dilute nitric acid and almost neutralized with ammonium hydroxide. Ferric chloride was then added in excess, followed by sufficient sodium carbonate to cause incipient precipitation, and finally just enough acetic acid to produce a clear solution. When this was poured into a large volume of hot water containing an excess of neutral ammonium acetate, a separation was effected, depending upon the insolubility of ferric phosphate in dilute acetic acid, and the tendency of dilute ferric acetate to form basic acetates in dilute hot solutions. As long as the reaction is acid, the alkali earth cannot be lost. The filtrates and washings were boiled with ammonium chloride, ammonium hydroxide, and ammonium carbonate; the washed precipitate of calcium

<sup>1</sup> See H. C. WOOD, JR.: *This journal*, 1898, i, pp. 83, 84.

and strontium carbonates was dissolved in weak nitric acid, concentrated and then examined spectroscopically. When a quantitative estimation was desired, the resulting syrup was evaporated several times to dryness with nitric acid, and the nitrates were separated by treatment with amyl alcohol (in which calcium nitrate alone is soluble). The residue of nitrate of strontium was dissolved in water, precipitated with sulphuric acid in the presence of 25 to 35 per cent alcohol, filtered on a Gooch crucible, washed, dried, and weighed. The above procedure differs from that employed by Wood in two points: (1) the fusion of the ash with sodium carbonate, — a process which was introduced at the suggestion of Professor H. L. Wells to eliminate the possibility of the loss of any strontium as sulphate; (2) the use of the more satisfactory separation with amyl alcohol in the final operation. In the examination of the urines it was found easier and equally satisfactory to precipitate the alkali earths directly by boiling with ammonium hydroxide and carbonate, and then to treat the precipitated salts alone.

In testing the delicacy of these methods for qualitative purposes, 10 mgm. of strontium in 300 c.c. of urine could easily be detected by any process. With smaller quantities, however, the fusion method seemed to give better results. In no case could 3 mgm. strontium be detected in 300 c.c. of urine. A quantitative test with the faeces was not made; the detection of small amounts of the metal in them must, however, be rendered less certain by the character of the materials present.

#### PROTOCOLS OF SOME EXPERIMENTS.

- I. A rabbit received a *subcutaneous injection* of 50 c.c. of approximately 4 per cent strontium acetate solution (containing 0.78 gm. metallic strontium by analysis). The urine and faeces were examined for strontium, with the results indicated in the table on page 11.

The results obtained with the urine correspond with those noted in man by H. C. Wood, Jr. The negative test recorded in one case with the faeces may have been due to the small amount of the latter collected at that period (34 hrs.), readily permitting a trace of strontium to escape notice.

Six days after the injection was made, the animal received a second one of 75 c.c. (containing 1.16 grams strontium). The urine was collected in three daily periods, and the rabbit then killed. A slight inflammation

was noted at the points of injection. The urine of the *first* day only gave a test for strontium. The element was, however, readily detected in the fæces of the *second* day. *Post mortem*, the *stomach* contents contained no strontium; the *intestinal contents* gave a very strong test for it.

Period after the first injection.	Urine.	Fæces.
1 hour. <sup>1</sup>	Faint spectroscopic trace.	
4 hours. <sup>1</sup>	Strong test.	
6 hours. <sup>1</sup>	Strong test.	Strontium detected.
6-21 hours.	Faint trace.	Strong test.
21-25 hours.	No strontium.	
25-34 hours.	"	Negative test.
34-48 hours.	"	
56-70 hours.	"	Strong test.
70-94 hours.	"	

<sup>1</sup> The urine was removed by catheter to prevent any contamination of the fæces.

II. A dog of 15 kilos, anæsthetized with A. C. E. mixture, received a *subcutaneous injection* of 100 c.c. of 4 per cent strontium acetate solution (containing 1.55 grams strontium). The animal was strongly affected by the injection; the urine contained increasing quantities of blood, and death occurred at the end of the fourth day. The subcutaneous tissue at the site of the injection was greatly inflamed.<sup>1</sup> Strontium was searched for as follows:

Day.	Urine.	Fæces.
1	Strong test.	No strontium.
2	" "	
3	Trace of strontium.	Strong test.
4	" "	

<sup>1</sup> The irritation at the seat of injection, in this and other experiments, corresponds with the local effects described by WOOD and ARNOLD (*loc. cit.*) after hypodermic injections in man and animals. They observed considerable local

The entire alimentary canal was examined, and strontium was found in the contents of the large intestine alone. The presence of strontium in the urine *after* the first day was not demonstrated in other experiments, — a fact possibly connected with the pathological conditions in the kidneys in this case.

- III. A cat received an *intraperitoneal* injection of 41 c.c. of 4 per cent strontium acetate solution (containing 0.64 gram strontium). The excretions were separately examined, in daily periods when possible, with the following results :

Period.	Urine.	Fæces.	Mixed urine and fæces.
After $\frac{1}{2}$ -hour.	No strontium.		
" 1 day.	Strontium in traces.		
" 2 days.	No strontium.		
" 4 days.	....	....	Strong test for strontium.
" 5 days.	....	....	" " " "
" 6 days.	....	....	Fair " " "
" 7 days.	....	Strontium present.	
" 8 days.	No strontium.		
" 16 days.			
" 17 days.	....	....	Slight " " "
" 18 days.			

Although a persistent diarrhœa rendered the separate collection of urine and fæces impracticable for several days, the failure to detect strontium in the urine of the second day and later indicates that the bowel formed the chief channel of excretion after that time. The slow elimination of the metal indicated by this experiment readily accounts for the small amounts present from day to day.

- IV. A dog of 9 kilos received (during A. C. E. anæsthesia) a *subcutaneous* injection of 25 c.c. 4 per cent strontium acetate solution (containing 0.38

pain and sloughing in one case, and were forced to discontinue this method of administration in man. Under such conditions, it is questionable whether the salt is completely, or even in part, absorbed. Unfavorable effects were avoided by us in our later experiments by the method of diffused injections described in Experiment VIII.

gram strontium). The animal was under observation for six days. Owing to recurrent diarrhœas uncontaminated urine was obtained (by catheter) on the second day only. It was free from strontium. Fæces collected free from any urine on the last four days gave strong tests for strontium; on the first day traces were observed in the fæces.

- V. A 10 kilo dog (anæsthetized with A. C. E. mixture) received 25 c.c. 4 per cent strontium acetate solution (containing 0.39 gram strontium) *subcutaneously*. The animal was killed at the end of thirty hours. The stomach contents contained no strontium; the intestinal contents gave a fair test; fæces collected after twenty-four hours yielded a good reaction.
- VI. A cat was given a *subcutaneous injection* of 20 c.c. strontium acetate solution (containing 0.31 gram strontium) during anæsthesia. The urine and fæces, obtained entirely separate, were analyzed quantitatively at intervals. Diarrhœa was largely avoided by a restricted diet of meat.

OUTPUT OF STRONTIUM.

Day.	Urine.	Fæces.
1-3	Unweighable trace.	
7	....	0.0164 gm. $\text{SrSO}_4$ = 0.0076 gm. Sr. <sup>1</sup>
13	....	0.0012 " " = 0.0006 " " <sup>1</sup>
18	....	0.0637 " " = 0.0304 " "
20	....	0.0366 " " = 0.0175 " "

<sup>1</sup> The figures for these two days are too low, owing to the inaccurate method followed, viz., precipitation of the sulphate in aqueous instead of alcoholic solution. Their only value consists in indicating the relative importance of the gut as an excretory channel for the strontium.

- VII. A dog of 10 kilos received *subcutaneously* 50 c.c. of the strontium acetate solution (containing 0.78 gram strontium) during anæsthesia. The animal was somewhat prostrated for a day or two; in view of the inflammation at the points of injection, it is by no means certain that all of the material injected was ever absorbed. During fourteen days the fæces were collected at intervals free from urine, and analyzed with the following results:

Day.	SrSO <sub>4</sub> .	Sr.
2	0.0314 gm.	0.0150 gm. <sup>1</sup>
4	0.0507 "	0.0242 " <sup>1</sup>
10	0.0331 "	0.0158 " <sup>1</sup>
13	0.2847 "	0.1359 "
14	0.0990 "	0.0473 "

<sup>1</sup> Here also the results are too low, owing to precipitation of the sulphate in aqueous solution, as explained under Experiment VI.

VIII. A dog of 6 kilos received *subcutaneously* during anæsthesia 15 c.c. of the strontium acetate solution, followed four days later by 20 c.c. of the same solution, — a total of 0.543 gram strontium. By introducing the solution in several (5) places, the local irritating action was avoided and no inflammation resulted.<sup>1</sup> A restricted diet of meat and lard was fed. The urine and fæces were collected separately, with practically no loss, for twenty-one days. The total urine during this period contained an unweighable trace of strontium; the total fæces contained

$$0.2092 \text{ gram SrSO}_4 = 0.0998 \text{ gram Sr.}$$

Unusually small amounts of calcium were found in both urine and fæces, an observation which suggested a possible explanation for the relatively small quantity of strontium recovered. Accordingly the next experiment was made.

IX. A dog of 14 kilos was given *subcutaneously* 18 c.c. of the strontium acetate solution (containing 0.28 gram strontium), the injection (during anæsthesia) being scattered at several places, as in Experiment VIII. The same diet also was fed, with the addition of one or two small bones. During seventeen days all fæces and urine were separately collected and examined. Strontium could not be detected in the urine. In addition to a very large amount of calcium, the fæces contained

$$0.498 \text{ gram SrSO}_4 = 0.237 \text{ gram Sr.}$$

<sup>1</sup> Compare the remarks in footnote 1, p. 11.



## IV.

The complete elimination of strontium takes place with such slowness that considerable interest is attached to the detection of the localization of this element in the tissues. Despite the earlier controversy on the subject,<sup>1</sup> it seems to be well established by the more recent observations of Cremer,<sup>2</sup> and Weiske<sup>3</sup> that after prolonged feeding of strontium salts, especially when added to a diet poor in calcium, strontium may accumulate in the bones. Under such conditions, Weiske found over four per cent of strontium oxide; like Raudnitz and Laborde, he also found traces of it in the liver and some other tissues. We have made an examination of parts of a few animals, with results as follows:

Animal.	Dose.	Time elapsed.	Strontium tests.
Cat.	0.39 gm. intravenously.	2 days.	Strong reaction with bone; test with muscle; none in blood.
Cat.	0.39 gm. subcutaneously.	19 hours.	Strong reaction with bone; none obtained from blood, muscle, liver, or brain.
Dog (II).	1.55 gms. subcutaneously.	5 days.	Trace in the entire liver.
Rabbit (I).	1.16 gms. subcutaneously.	3 days.	None in the liver.

J. B. MacCallum<sup>4</sup> has demonstrated that the so-called saline purgatives are effective after intravenous or subcutaneous injection, as well as when they are introduced directly into the intestinal tract. Barium chloride is particularly active and, like the others, gives rise to an increased secretion of fluid into the intestine. The peristaltic movements produced can be suppressed by the application of solutions of calcium, magnesium or strontium chloride. Since all these salts are most efficient when they reach the intestinal wall by way

<sup>1</sup> Cf. Jahresbericht für Thierchemie, 1874, iv, p. 317, for references to the literature.

<sup>2</sup> CREMER: Sitzungs-Berichte der Gesellschaft für Morphologie und Physiologie in München, 1891, vii, p. 124.

<sup>3</sup> WEISKE: Zeitschrift für Biologie, 1894, xxxi, p. 421.

<sup>4</sup> MACCALLUM: This journal, 1903, x, p. 101; 1904, x, p. 263.

of the circulation, it seems reasonable to assume a functional connection between the physiological action of the salts on the gut, and their elimination by passage (secretion) through the walls of the intestinal tract. For calcium, magnesium and strontium salts, this path of elimination has now been demonstrated. Whether, as seems probable, barium salts are likewise excreted by the bowel, we hope soon to ascertain definitely; their presence in the saliva has been shown by Neumann.<sup>1</sup>

#### V. SUMMARY OF THE MORE IMPORTANT OBSERVATIONS.

Strontium salts are eliminated to a relatively small extent only by the kidneys, even after direct introduction into the circulation. The excretion in the urine begins soon, and ceases usually within twenty-four hours.

The larger portion of the strontium eliminated is found in the fæces, whether the introduction of the element be per os, subcutaneously, intravenously, or intraperitoneally.

The place of excretion is apparently restricted to the region of the alimentary tract beyond the stomach. A functional relation to certain phenomena of intestinal peristalsis, etc., is suggested.

The rate of elimination is slow, and is apparently influenced by the calcium-content of the food.

Strontium is found deposited in the body chiefly in the bones; traces may be met with in the liver and muscles.

<sup>1</sup> J. NEUMANN: *Archiv für die gesammte Physiologie*, 1885, xxxvi, p. 576.

# NICOTINE TOLERANCE IN RABBITS, AND THE DIFFERENCE IN THE FATAL DOSE IN ADULT AND YOUNG GUINEA-PIGS.

By ROBERT A. HATCHER.

[*From the Laboratory of Pharmacology of the Medical College of Western Reserve University.*]

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## I. INTRODUCTORY.

**D**URING the course of a series of experiments undertaken to determine whether the serum of rabbits which had been rendered tolerant to nicotine, would protect guinea-pigs against an ordinarily fatal dose of nicotine, some interesting facts in regard to toxicity were observed. The primary object of the research was not attained, however, owing in part to the difficulty of rendering rabbits sufficiently tolerant, and in part to the inability to fix the fatal dose of nicotine in guinea-pigs with sufficient exactness.

In the earlier experiments upon both rabbits and guinea-pigs pure nicotine in one per cent solution was used, but, as this is strongly alkaline and its injection painful in nearly all the experiments, the alkaloid was converted into the sulphate by the addition of deci-normal sulphuric acid. In every case the dose is calculated in pure nicotine, and is expressed in milligrams per kilogram of body weight, one per cent solutions being employed by hypodermic injection.<sup>1</sup>

<sup>1</sup> BLYTH ("Poisons, Their Effects and Detection," 1885, i, p. 243) states that forty-nine parts of sulphuric acid correspond to one hundred and sixty-two parts of nicotine; HAGER ("Pharmaceutische Praxis," 1900, ii, p. 477) gives half this

## II. PICTURE OF POISONING.

The first symptoms following the hypodermic injection of a fatal dose of nicotine in the rabbit usually appear within five minutes. There are swaying movements of the body, caused by loss of co-ordination. The ear-vessels are dilated, but may be caused to contract

TABLE I.  
RECORD OF NICOTINE  
The black figures at the head of columns indicate the day of  
R and S were used merely to observe

Animal.	1	2	3	4	5	6	7	9	10	11	12	13	14	15	16	18	19	20	21
A	10	..	12.5	..	15.0	..	20												
B	10	..	15.0	..	20.0	..	25												
C	12	..	15.0	..	20.0														
D	10	..	15.0	..	17.5	..	20	22.5	..	25	30.0	..	35	..	35.0	35	..	36.5	..
E	10	..	15.0	..	17.5	..	20	..	20	..	22.5	..	25	..	27.5				
F	10	..	15.0	..	17.5	..	20	20.0	20	20	25.0	26.5							
G	10	..	15.0	..	17.5	..	..	22.5											
H	15	20	23.0	24															
K	15	..	17.5	..	20.0	..	..	..	20	..	..	..	..	..	25.0	..	..	..	20
L	15	..	..	..	15.0	..	..	..	15	..	..	..	..	20	..	..	20	..	..
M	15	..	15.0	..	15.0	..	..	..	15	..	..	..	..	20	..	..	20	..	..
N	15	..	..	18	..	..	21	..	24	..	..	27	..	30	..	..	..	..	..
O	15	..	..	..	19.0														
P	10	..	..	..	15.0	..	..	..	..	..	20.0	..	..	22	..	..	..	..	..
Q	15	16	17.0	18	19.0	20	46												

instantly by slight stimulation, such as blowing upon the body. This reaction is lost when the poisoning is more advanced, the vessels remaining dilated. The animal rests upon the belly, the legs outstretched, the nose gradually approaching the floor, and then the head

amount of acid. According to the figures of Blyth, the nicotine used in my experiments, the purest obtainable in ordinary commercial channels, was ninety-seven per cent of absolute nicotine.

tending to rest upon the cheek; about this time clonic convulsions occur, affecting the fore or hind limbs, or general in character. The respiration becomes slower, and ceases before the heart stops.

The pupil-effects are not constant; the pupils may be dilated or very much contracted. Defecation does not usually occur when death is early, and the same may be said of urination; violent peris-

TABLE I.

## INJECTIONS INTO RABBITS.

observation; the figures in the columns the dose (mg. per kg.).

symptoms and effect of heat and cold.

	22	23	25	26	27	28	29	33	37	39	43	44	50	55	61	85	87	89	91	92	93	94	95	96
36.5	..	..	..	..	..	36.5	..	40																
..	..	20	..	..	..	..	20	20	..	20	25	..	25	..	30	..	..	..	15	30	30	31	32	33
..	20	..	..	20	..	..	..	20	20	..	..	20	..	25	..	10	25	27	29	..	31			
..	20	..	..	20																				
32.5																								
27.5	..	..	30																					

taltic movements of the intestines may nearly always be seen through the intact skin after death. After doses just fatal, death commonly results within an hour and a half; in nearly fatal cases the animal becomes practically normal in from two to three hours.

Artificial respiration was tried in a number of cases where death was evidently imminent, but in no case did it save the animal. Marked improvement was seen in several cases where artificial heat was applied. This was strikingly shown in the cases of rabbits

R and S. The room-temperature being about five degrees C. they were kept warm by placing them near a large can filled with hot water. During the first forty minutes of the experiment S did not exhibit symptoms of extreme intoxication, whereas R did so almost from the beginning. Owing to a slight delay in refilling the can when it became cooled, both rabbits were somewhat chilled, and immediately showed signs of great depression; upon reapplying heat, R began to improve at once, but S died about twenty minutes later, just an hour from the beginning of the experiment; but for this chilling there is but little doubt that recovery would have followed.

When the animal rests with wide-stretched legs, nose to the floor, and head inclined toward the side, with the respiration slowed, it may be taken as certain that the dose is not very far from the fatal amount.

In guinea-pigs the symptoms do not vary so much with the size of the dose, and it is, therefore, frequently impossible to state whether the dose was very nearly fatal or not.

After the hypodermic injection of a fatal or nearly fatal dose of nicotine in the guinea-pig, salivation and rapid chewing are noticed within one minute, convulsions occur in from one-half to three minutes, the animal falling upon its side and executing rapid pawing movements of the forelegs for half a minute or more. There is extreme opisthotonos. A greenish mucus is commonly forced from the mouth. The respiration ceases during the convulsion, or permanently in some cases, suggesting the thought that the mucus might cause closure of the epiglottis, none being found in the trachea, however, in such fatal cases. The frequency with which comparatively small doses caused death at this stage suggests some such cause.

Almost invariably after the rotary movements of the forelegs cease, the animal rolls over a number of times, sometimes for a distance of two feet. Death results from failure of respiration, the heart continuing to beat for an hour or more after respiration ceases, the heart being exposed. The fatal dose for an adult guinea-pig is from forty to forty-five milligrams per kilogram; that for the rabbit being approximately twenty milligrams per kilogram.

### III. ACQUIRED TOLERANCE TO NORMALLY FATAL DOSES IN RABBITS (SEE TABLES I AND II).

Rabbits acquire a limited tolerance, but very slowly. D acquired a tolerance amounting to about fifteen milligrams per kilogram of body

weight in thirty-three days, during which time fourteen doses were given. This animal showed less effects from the second dose, fifteen milligrams per kilogram, than any of the other rabbits of the series; but twenty-five milligrams per kilogram on the eleventh day was not very far from fatal.

TABLE II.  
FATAL DOSE OF NICOTINE FOR TOLERANT RABBITS.

Rabbit.	Fatal dose.	Under observation.	No. of doses.	Remarks.
	mg. per kg.	days.		
A	20.0	7	4	
B	25.0	7	4	
C	20.0	5	3	
D	40.0	33	14	
E	27.5	16	8	
F	26.5	13	9	
G	22.5	9	5	
H	24.0	4	4	
K	survived 33.0	96	18	Bled to death after surviving 33 days; 30 days elapsed between 13th and 14th doses.
L	31.0	93	16	30 days elapsed between 11th and 12th doses; alternate days thereafter.
M	20.0	7	7	
N	survived 32½	2	7	
O	19.0	4	2	
P	30.0	25	7	
Q	survived 20.0	6	6	46 mg. per kg. administered by mistake.
R	„ 20.0	1	1	
S	25.0	1	1	

A single dose confers very little tolerance, even after some time: nineteen milligrams per kilogram proved fatal to O, four days after having had the first dose of fifteen milligrams per kilogram.

Tolerance, when once established, is retained for some time. Thirty milligrams per kilogram in K, and twenty-five milligrams per kilogram in L, were not very nearly fatal after an interval of a month.

Tolerance appears to remain nearly stationary when the drug is withdrawn; L withstood twenty-five milligrams per kilogram, the same

dose given a month previously, very well, but died some days later when the dose was raised to thirty-one milligrams per kilogram.

Table I gives the dose, the days of administration, and the results.

Table II gives the fatal dose, the number of days under observation and the total number of doses given.

#### IV. THE BEST METHOD OF INDUCING TOLERANCE.

Cushny<sup>1</sup> states: "If a quantity (of nicotine) just smaller than the lethal dose be injected into an animal, and two or three days after its recovery a second injection of the same amount be made, death will follow, but some of the symptoms, such as convulsions, may be entirely absent." This does not seem to apply to rabbits. Rabbit F received twenty milligrams per kilogram on the seventh day, and the following note occurs in the record—"Evidently, very nearly fatal," yet, upon the ninth, tenth, eleventh, and twelfth days doses of twenty and twenty-five milligrams per kilogram were survived. Rabbit D received twenty-five milligrams per kilogram on the eleventh, as previously noted, this dose being not very far from lethal; but this rabbit survived thirty milligrams per kilogram the next day, a dose *very* nearly fatal. Rabbits H and Q similarly survived daily doses near the lethal for three and five days respectively, while rabbit K received nearly fatal doses for five days and was finally bled to death.

When doses approaching the fatal are given at shorter intervals than three days, the rabbits usually lose weight after about three weeks. The weight increases again if the drug is discontinued for a time, and, as previously stated, the acquired tolerance is retained.

In two instances, rabbits K and N, ulcers appeared, not at the site of injection, but three to four inches distant, near the base of the spinal column. In one case this disappeared when the drug was withdrawn and reappeared when it was resumed.

From these experiments we may conclude that tolerance is best established by giving large doses at intervals of about three days with occasional intermissions of a week or ten days; rather than by the frequent repetition of moderately large doses. Rabbits K, L, and M had doses of fifteen to twenty milligrams per kilogram for a month, at intervals of three to four days, without acquiring any considerable degree of tolerance, much less than that acquired by D in thirty-three

<sup>1</sup> CUSHNY'S Pharmacology, 1899, p. 266.



days by large doses, K barely surviving thirty, while L succumbed to thirty-one milligrams per kilogram (M is disregarded since it died during the night after apparent recovery from a small dose).

Dr. Torald Sollmann, in a series of observations upon similar lines, observed that rabbits are less affected by a given dose after it has been repeated a number of times, showing an acquired tolerance to doses just under the lethal. My own observations confirm this, but my experiments did not afford so good an opportunity for observing this effect.

#### V. THE INFLUENCE OF AGE UPON SUSCEPTIBILITY IN THE GUINEA-PIG.

The attempt to fix the fatal dose of nicotine in guinea-pigs gave results varying widely, particularly in those cases in which largely increased doses were given after an intermission of three weeks.

That the lessened susceptibility in these cases was not due to tolerance acquired from a single dose is shown by experiments upon Nos. 36 and 37 (see Table III).

The suggestion naturally presents itself that age has some influence upon susceptibility, and in order to investigate the matter further, a number of very young guinea-pigs, weighing from one hundred and ten to two hundred grams each, were injected with doses varying from ten to twenty-five milligrams per kilogram. From a study of the table and the symptoms recorded, it may be stated that the average fatal dose for a guinea-pig of one hundred and fifty grams weight is from eighteen to twenty milligrams per kilogram (about the same proportion to weight as for adult rabbits); while adult guinea-pigs, weighing four hundred grams, require about twice that proportion, or about forty milligrams per kilogram.

The smallest fatal dose was fourteen milligrams per kilogram, for a two-hundred-gram guinea-pig (No. 9); while twelve milligrams per kilogram proved all but fatal to No. 25, weighing one hundred and eleven grams, and to No. 37, weighing a hundred and fifty grams. The largest dose survived by one of two hundred grams or under was twenty-five milligrams per kilogram (No. 30). As the weight increases, we find the smallest fatal dose, where the weight is three hundred grams or over, is more than thirty milligrams per kilogram, if we except No. 12, weighing three hundred and seventy grams, which died from a dose of nineteen milligrams per kilogram, while No. 20 (four hundred grams) survived a

TABLE III.<sup>1</sup>  
EXPERIMENTS WITH NICOTINE UPON GUINEA-PIGS.

Experiment.	Weight in grams.	Dose.	Remarks.
Not nearly fatal.			5 c.c. of serum of tolerant rabbit mixed with dose.
40 <sup>2</sup>	150	18 <sup>2</sup>	
S	200	10	
Nearly fatal.			<p>Received 5 c.c. of serum of tolerant rabbit 24 hours earlier.</p> <p>Received 5 c.c. of serum of tolerant rabbit 24 hours earlier.</p> <p>Same animal as No. 5, used 20 days previously.</p> <p>Used 20 days previously.</p> <p>" 21 " "</p> <p>" 34 " "</p> <p>" 34 " "</p> <p>" 34 &amp; 14 " "</p>
25	111	12	
26	135	10	
37	150	12	
30	160	25	
27	170	14	
2	173	20	
38	175	18	
39	190	20	
36	200	15	
33	210	18	
28	220	20	
14	267	28	
17	275	18	
19	330	30	
20	340	30	
22	410	40	
21	425	33	
Fatal.			<p>Used 3 days previously (No. 25).</p> <p>" 3 " " (No. 26).</p> <p>Second dose 7 days after first.</p>
31	110	25	
32	140	20	
29	150	22.5	
37	170	18	

<sup>1</sup> The animals in each class are arranged in the order of their weights.

<sup>2</sup> See text for explanation of apparent protection.

TABLE III.—*Continued.*

Experiment.	Weight in grams.	Dose.	Remarks.
Fatal.			Second dose 7 days after first.
13	197	15	
9	200	14	
35	200	15	
36	200	25	
4	202	20	
6	210	18	
3	220	22	
7	220	16	
41	255	21	
12	370	19	
24	400	45	
Much more than just fatal.			5 c.c. of serum of tolerant rabbit mixed with dose.
1	180	35	
34	200	35	Third dose in 70 days.
Not classified.			
10	195	12.5	Used 20 days previously.  Used 20 days previously (No. 11).
11	213	13.5	
16	265	16	
18	335	20	

dose of forty-five milligrams per kilogram for sixteen minutes, and No. 22 (four hundred and two grams) recovered from a dose of forty milligrams per kilogram.

From this we must conclude that the toxicity of nicotine is fully twice as great for very young guinea-pigs as it is for adults.

Table III gives the results of forty experiments upon guinea-pigs.

## VI. HAS THE SERUM OF TOLERANT RABBITS ANY PROTECTIVE POWER FOR GUINEA-PIGS?

When the work in this laboratory had been brought to the present stage, it was learned that Prof. J. McFarland, whose work was unpublished, had obtained negative results in the effort to obtain an antitoxic serum for nicotine.

While the results of the present series of experiments are not conclusive, they cannot be said to be very encouraging.

Hirschlaff's<sup>1</sup> report of the brilliant results obtained by the use of an antitoxic serum obtained in an analogous manner for morphine has been completely disproved by J. Morgenroth.<sup>2</sup>

The failure to produce such antitoxic serum with morphine, while, of course, not conclusive, nevertheless points to a similar result with nicotine. The results in guinea-pigs Nos. 40 and 41 can be accounted for by the fact that the nicotine was given in much less concentration (Meltzer<sup>3</sup> having shown that strychnine is much less toxic in very dilute solutions), and by the lessened rate of absorption due to proteids, which also lessens toxicity, as shown by the author<sup>4</sup> in the case of strychnine.

In the cases of Nos. 38 and 39, which had 5 c.c. of the serum twenty-four hours earlier, while both recovered from doses of nicotine which might reasonably be expected to prove fatal, we find that No. 2 and No. 30 received twenty and twenty-five milligrams per kilogram, respectively, and, further, the serum did not prevent convulsions; hence the protection, if any, was slight.

## VII. CONCLUSIONS.

Nicotine is very uncertain in its action. While death is due to failure of respiration, artificial respiration, if effective at all, must be begun before the centres show evidence of marked depression. The stimulating effects of heat are undoubtedly useful in combating the toxic effects.

No cumulative effects can be seen when nicotine is given daily for several days. Nutritional disturbances follow its prolonged use, as is

<sup>1</sup> HIRSCHLAFF, L.: *Berliner klinische Wochenschrift*, 1902, xxxix, p. 1174.

<sup>2</sup> MORGENROTH, J.: *Berliner klinische Wochenschrift*, 1903, xl, p. 471.

<sup>3</sup> MELTZER, S. J.: *Journal of experimental medicine*, 1900-1901, v, p. 643.

<sup>4</sup> HATCHER, R. A.: *American journal of pharmacy*, 1902, lxxiv, p. 283.

indicated by the constant loss of weight after a time, and by the appearance of ulcers at a point some distance from the seat of injection, disappearing with the cessation of its use, and reappearing upon resumption.

The preparation of an antitoxic serum for nicotine is not practical, if possible, with our present knowledge.

The average fatal dose of nicotine for an adult rabbit is approximately twenty milligrams per kilogram; for the adult guinea-pig the average fatal dose is approximately forty milligrams per kilogram, the very young guinea-pig being much more susceptible, the average fatal dose for one of a hundred and fifty grams or less being about fifteen milligrams per kilogram.

I am greatly indebted to Dr. Torald Sollmann for suggestions and assistance in carrying out this research.

# STUDIES ON THE "PARADOXICAL" PUPIL-DILATION CAUSED BY ADRENALIN. I.—THE EFFECT OF SUBCUTANEOUS INJECTIONS AND INSTILLATIONS OF ADRENALIN UPON THE PUPILS OF RABBITS.

BY S. J. MELTZER AND CLARA MELTZER AUER.<sup>1</sup>

[From the Rockefeller Institute for Medical Research.]

IN our studies of the effect of intravenous injections of adrenalin upon the blood-vessels of rabbits' ears,<sup>2</sup> we made the observation that section of the cervical sympathetic nerve changes this effect in some respects: the constriction of the vessels of the corresponding ear sets in later, develops gradually, reaches the maximum later, and lasts incomparably longer than in the ear of a normal animal. In further studies upon subcutaneous injections,<sup>3</sup> the influence of the section of the sympathetic nerve was still more marked. While in a normal animal a moderate dose of adrenalin has either no effect, or the effect is rather a marked dilatation of the blood-vessels, a subcutaneous injection of such a dose of adrenalin into a rabbit in which the sympathetic nerve is cut or resected, effects invariably a long-lasting constriction of the blood-vessels of the ears.

Section of the sympathetic nerve, then, has a distinctly modifying influence upon the vasomotor effect of the extract of the suprarenal gland, at least as far as the ear-vessels are concerned. The question presented itself, whether section of the sympathetic nerve could also influence the effects of the suprarenal extract upon other organs which stand under its control. Such an organ is the pupil. Lewandowsky<sup>4</sup> discovered that intravenous injection of suprarenal extract causes a dilatation of the pupil. This was confirmed by Boruttau<sup>5</sup> and by

<sup>1</sup> Research scholar of the Rockefeller Institute for medical research.

<sup>2</sup> MELTZER and MELTZER: This journal, 1903, ix, p. 147.

<sup>3</sup> MELTZER and MELTZER: This journal, 1903, ix, p. 252.

<sup>4</sup> LEWANDOWSKY: Archiv für Physiologie, 1899, p. 360.

<sup>5</sup> BORUTTAU: Archiv für die gesammte Physiologie, 1899, lxxxviii, p. 112.

Langley.<sup>1</sup> The effect is very brief, it lasts less than a minute. It is distinct in cats, is less pronounced in rabbits. All agree that a subcutaneous injection has no effect, and this is explained by the assumption that the extract, before it reaches the blood, is oxidized in the lymph spaces.

In extensive experience with subcutaneous injection of adrenalin in rabbits, we can confirm the observation that in normal animals the pupils are never affected by an injection of even a large dose, provided it does not cause asphyxia of the animal. It is different, however, with operated animals. In our studies of the effect of subcutaneous injections of adrenalin upon the vasomotor condition of the ear-vessels, during which, in a large number of rabbits, the sympathetic nerve was cut, or the superior cervical ganglion was removed, it was found that in many cases the pupil became dilated on the operated side after a subcutaneous injection; but the effect seemed to be inconstant. An analysis of our notes brought to light, however, that the phenomenon was observed only in those animals in which the ganglion was removed, and when the injection was made at least twenty-four hours after its removal. To test this point we instituted a series of experiments on rabbits in which the relations of the excision of the ganglion and the cutting of the sympathetic nerve to the effect upon the pupil of a subcutaneous injection of adrenalin were studied systematically. The results we now obtained were constant and perfectly uniform. They are, briefly stated, as follows:—

After section of the sympathetic nerve, a subcutaneous injection of even a large dose of adrenalin has no effect on the pupil. This can be said to be the rule. In a very few exceptions the injection seemed to cause a slight dilatation. It was, however, under all circumstances very slight, lasting only a few seconds, and in fact, we were never very sure of its occurrence.

When, however, the superior cervical ganglion was excised, the effect of a subcutaneous injection of adrenalin upon the pupil was a very striking one. Even after the injection of such a moderate dose as 0.6 c.c., the pupil on the side on which the ganglion was removed became dilated *ad maximum*. It is safer, however, to employ a somewhat larger dose,—about 1.0 or 1.2 c.c. of adrenalin chloride (1:1000). In ten or fifteen minutes the pupil then becomes very dilated, and remains so for at least one hour, and very often for

<sup>1</sup> LANGLEY: Journal of physiology, 1901-1902, xxvii, p. 237.

more than even two hours. In some cases the dilatation begins to show three to four minutes after the injection.

When the dilatation caused by adrenalin is *ad maximum*, the dilated pupil does not react to light.

When previous to the administration of adrenalin the pupils were made myotic by means of instillation of eserine, the subcutaneous injection of adrenalin caused, nevertheless, an *ad maximum* dilatation of the pupil on the operated side, while on the normal side the myosis was not affected in the least. Adrenalin apparently overcomes completely the effect of eserine.

The dilating effect of adrenalin upon the pupil can be seen only when the subcutaneous injection is made at least twenty-four hours after the removal of the ganglion. Injections made at an earlier period exercise apparently no effect upon the pupil, — while the vaso-constricting effect upon the ear-vessels of the operated side is very distinct, even if the injection be made soon after the operation.

The effect upon the pupil takes place only when the entire ganglion is removed. Section of all the connecting branches except the upper one, or removal of the lower two-thirds of the ganglion, does not lead to a response of the pupil by dilatation after a subcutaneous injection of adrenalin. In tearing out the ganglion in earlier experiments, we met with a few failures. On reopening the wound it was found that the removal was incomplete. The subsequent removal of the remaining portion brought out the usual result.

We have also studied the effect upon the pupil of instillation of adrenalin into the conjunctival sac after removal of the ganglion. Radziewsky<sup>1</sup> was the first to state that instillation of suprarenal extract exerts no effect upon the pupil. This was confirmed by Lewandowsky<sup>2</sup> and Boruttau.<sup>3</sup> From our studies we can confirm the observation that instillation does not affect the pupil of a normal rabbit. Neither does it cause any change in the pupil even after the sympathetic nerve is cut. However, twenty-four hours after complete excision of the superior cervical ganglion, instillation of adrenalin causes a dilatation of the pupil on the operated side. The degree of the dilatation depends upon the quantity instilled. After four or five instillations

<sup>1</sup> RADZIEWSKY: Berliner klinische Wochenschrift, 1898, p. 572.

<sup>2</sup> LEWANDOWSKY: *Loc. cit.*

<sup>3</sup> BORUTTAU: *Loc. cit.*



of two drops at a time at intervals of two or three minutes, the dilatation can be *ad maximum*. The dilatation lasts also for some time—several hours; usually, however, it disappears sooner than after a subcutaneous injection. The myotic effect of eserin is also overcome by instillation of adrenalin. We have never observed constitutional effects from instillation; for instance, we have never seen a constriction of the ear-vessels taking place after otherwise effective instillations of adrenalin.

The difference in the effect between section or resection of the sympathetic nerve and excision of the superior cervical ganglion is brought out best when the ganglion is removed on one side and the sympathetic nerve cut on the other side of the same animal. After a subcutaneous injection of a medium dose of adrenalin, there is then no difference in the condition of the ear-vessels on the two sides, both ears look equally pale; but there is a striking contrast in the pupils, the pupil on the side without the ganglion is widely dilated, while the one on the other side remains more contracted than normal, on account of the section of the sympathetic nerve.

We have frequently employed the following method to bring out some of the above-described results in a striking manner. The hind leg of an operated rabbit was tightly ligated above the knee and a medium dose of adrenalin injected into the leg peripheral to the ligature. When after some time the ligature was removed, the pupil on the side on which the ganglion was removed became widely dilated in less than half a minute, while on the normal side or the side on which only the sympathetic nerve was cut the pupil remained unchanged. It required some experience to attain the proper degree of tightness of the ligature. In a rabbit it is quite difficult to apply a single ligature tight enough so that none of the adrenalin shall escape under the ligature. We then often see a slight dilatation of the pupil (and constriction of the ear-vessels) taking place before the ligature is removed. A double ligature will safely prevent the escape. But then the ligature has to be removed in less than an hour; otherwise the absorption becomes impaired and also frequently leads to gangrene of the leg. However, after some experience it is not difficult to find a proper degree of tightness (best two ligatures of moderate tightness) which will prevent any escape and which at the same time will not permanently impair absorption. In such a case, the ligature can be kept for two hours and even longer, and after its removal the effect of adrenalin is a striking and nearly an instan-

taneous one. We shall discuss later the significance of such an experiment.

Our experience with the effects of intravenous injections of adrenalin on the pupil, is still too scanty to permit any detailed statement; we gathered it only incidentally in experiments carried on for other purposes. As far as our experience goes, intravenous injections showed the same conditions as were observed in the subcutaneous injections and instillations, *i. e.*, on the side on which the ganglion was removed the pupil also became dilated after an intravenous injection, and the dilatation lasted for quite a long time; whereas on the normal side or on the side on which the sympathetic nerve was cut, the dilatation was comparatively insignificant and lasted only a few seconds. We may, however, come back to this subject in a later communication.

We shall illustrate the above-stated results by a few protocols of experiments. They are greatly abbreviated, as many of these experiments were utilized also for a study of the vasomotor phenomena in the ears.

*Experiment 53. Dec. 1, 1902.*—Large brown rabbit.<sup>1</sup> Right ganglion torn out, . . . left side normal. . . .

*Dec. 2.*— . . . No difference in size of the two pupils.

*Dec. 3.*—4.00 P. M.: Subcutaneous injection of 1.5 c.c. of adrenalin (1 : 1000), right pupil much dilated, no noticeable effect upon left. 5.30, 7.00, and 9.00 P. M.: No change.

*Dec. 4.*—10.00 A. M.: Both pupils about equal. 2.45 P. M.: Injected subcutaneously 1.5 c.c. 2.50: Right pupil large. . . . 3.10: Right pupil very wide. . . . 4.40 and 6.00 P. M.: Right pupil wider than left. 9.00 P. M.: Right pupil same size as left.

*Dec. 12.*—Right pupil smaller than left.

*Dec. 18.*—Right pupil smaller than left. Subcutaneous injection of 1.5 c.c. . . . Right pupil much dilated.

*Dec. 23.*—8.00 P. M.: Subcutaneous injection of 0.6 c.c. In a few minutes right pupil wide. 9.00 P. M.: Right pupil smaller again, but still wider than before injection.

*Dec. 24.*—Hind leg ligated, injected below ligature 1.2 c.c. of adrenalin, . . . "ligature taken off in five to seven minutes, the right ear became pale immediately, the left ear still full; the right pupil very wide."

In this experiment repeated injections invariably brought out a prompt effect, — even a dose of only 0.6 c.c. caused an unmistakable

<sup>1</sup> All animals were narcotized during operation.

dilatation. With doses of 1.5 c.c. the dilatation lasted at least three hours.

*Experiment 61.* — Small gray rabbit. . . .

*Dec. 9.* — Cervicals operated.

*Dec. 16.* — Wound reopened and left ganglion removed. . . . 8.30 P. M. : Left pupil smaller. . . . Injected subcutaneously 1.5 c.c. Right ear, no change ; left ear pales gradually ; no effect on eyes.

*Dec. 17.* — 8.30 P. M. : . . . left pupil a trifle wider than right. Injected subcutaneously 1.5 c.c., . . . in a few minutes left pupil wider than right.

*Experiment 62.* *Dec. 10.* — Right sympathetic cut.

*Dec. 16.* — Right pupil a trifle smaller than left. 11.45 A. M. : Injected subcutaneously 1.5 c.c. . . . No effect on eyes. 4.15 P. M. : Right pupil a trifle smaller. Wound reopened and right ganglion torn out. . . . 6.00 P. M. : Right pupil smaller. . . . 8.00 P. M. : Injected 1.5 c.c. ; right ear becomes pale ; . . . no effect on eyes ; . . . a second injection : no effect on pupils.

*Dec. 17.* — 6.00 P. M. : Pupils about equal. Injected subcutaneously 1.5 c.c. . . . In three minutes the right pupil begins to dilate, in fifteen minutes very wide. . . . 9.45 : Right pupil much wider than left.

*Dec. 23.* — 6.00 P. M. : Hind leg tightly tied with Esmarch bandage ; injected into distal end 2.0 c.c. ; toxic symptoms while bandage still on ; bandage removed ; . . . right pupil very wide. 9.00 P. M. : Right pupil wide.

*Dec. 24.* — Injected subcutaneously 1.00 c.c. Right pupil becomes wide.

*Jan. 14, 1903.* — 5.30 P. M. : Leg very firmly tied. No effect noticed. Ligature removed at 7.00 P. M. In two minutes pupil very wide.

In this experiment section of the sympathetic had no effect ; but twenty-four hours after subsequent removal of the ganglion, the effect was invariably positive. It shows also that even a firmly applied Esmarch bandage permitted the escape of the adrenalin.

*Experiment 67.* *Jan. 13, 1903.* — 5.30 P. M. : Right ganglion removed ; . . . right pupil much smaller than left.

*Jan. 14.* — Evening. Instilled into both conjunctival sacs adrenalin. In about five minutes the right pupil began to dilate slowly. . . . In three hours still widely dilated. No effect on left pupil.

*Experiment 70.* *Jan. 27, 1903.* — Large rabbit. Right sympathetic resected ; after ten minutes right pupil smaller than left.

*Jan. 30.* — Injected subcutaneously 1.2 c.c. adrenalin ; . . . effect on ear-vessels but not on pupils.

*Jan. 31.* — 11.30 A.M. : Left ganglion removed, . . . pupils about equal, left conjunctiva injected. 6.00 P.M. : Instilled adrenalin into both conjunctival sacs, no result.

*Feb. 1.* — 11.00 A.M. : Left pupil somewhat wider than right. Instilled adrenalin into both conjunctival sacs ; right pupil, no change, left dilates slowly ; after three-quarters of an hour, well dilated ; no reaction to light.

*Feb. 2.* — Leg tied with double ligature ; injected 1.5 c.c. adrenalin peripherally to ligature ; apparently no escape ; ligature removed after two and a half hours ; twenty-five seconds later left pupil widely dilated ; no change in right pupil.

*Experiment 77. March 2.* — Right ganglion removed, left sympathetic resected.

*March 4.* — Instilled adrenalin into both conjunctival sacs. In two minutes right pupil rapidly dilating ; in ten minutes dilated ad maximum ; no change in left pupil.

These few protocols are sufficient to illustrate the essential points of our statements. But we have made quite a large number of such experiments, and all gave uniformly the same results. In nearly a dozen instances the experiments were demonstrated to interested observers without a single failure. The previous use of eserine brings out the main phenomenon in a striking way.

The longest period during which we had operated animals under observation was three and one-half months. The effect of a subcutaneous injection was at the end of this period as pronounced as in the first few days after operation. The effect of instillations seemed somewhat diminished, the dilatations did not reach the maximum, and returned to normal sooner than before. However, these observations have not been numerous enough as yet to permit positive conclusions to be drawn.

Our protocols of the above-mentioned series of experiments contain a few notes with reference to the effects of section of the sympathetic nerve and removal of the ganglion upon the condition of the pupil, which may deserve to be recorded.

In a few cases the ganglion was removed a few days after the sympathetic had been resected. In none of these cases was the constriction of the pupil increased by the removal of the ganglion. Furthermore, when the sympathetic nerve was cut on one side, and at

the same time the ganglion was removed on the other side, the constriction of both pupils was, in our experiments, the same. Budge,<sup>1</sup> Langendorff,<sup>2</sup> and others state that on the first day after the operation, the pupil on the side on which the ganglion was removed is more constricted than on the side on which the sympathetic was cut, and the conclusion is therefore drawn that besides the dilating tonus, kept up by the fibres of the sympathetic nerve, an additional dilating tonus emanates from the cells of the superior cervical ganglion. Kowalewsky<sup>3</sup> and other writers, however, could not confirm this statement. Our own restricted experience seems to agree with that of the latter authors.

In most of the experiments on the removal of the ganglion, we find that on the day after the removal, the pupil on the operated side was wider again, — being mostly “about equal” to the pupil of the other side, if the latter was normal, or “a trifle wider” when the sympathetic was cut on the other side. Similar observations have been already recorded by Budge,<sup>4</sup> and were subsequently observed by a number of other writers. We find in our notes, however, that in some cases, from the seventh day on after the operation, the pupil on the ganglion side was smaller again than on the normal side (see above, Experiment 53). In others the conditions seemed to vary during the entire period of observation, sometimes the pupil on the ganglion side being equal to that of the sympathetic side, and at other times being a trifle larger, without there being any visible reason for the variations.

We have never noticed that excitement of the animal favored a dilatation of the pupil on the ganglion side. Neither have we observed that the usual state of even deep anæsthesia (we employed only ether) brought out a greater dilatation of the pupil on the ganglion side. In two cases, however, in which the very deep anæsthesia brought on asphyxia of the rabbits, the pupil on the ganglion side was noticed to be visibly wider than on the sympathetic side; the latter also became somewhat dilated.

In a number of protocols of experiments in which the ganglion was removed, it was noted that the conjunctiva on the operated side was

<sup>1</sup> BUDGE: Ueber die Bewegung der Iris, Braunschweig, 1855, p. 125.

<sup>2</sup> LANGENDORFF: Klinische Monatsblätter für Augenheilkunde, 1900, xxxviii, p. 129 and p. 823.

<sup>3</sup> KOWALEWSKY: Archives slaves de biologie, 1886, i, p. 592.

<sup>4</sup> BUDGE: *Loc. cit.*

injected. There is no such remark in any case in which the sympathetic was cut.

In three rabbits the sympathetic nerves were stimulated low down in the neck many weeks after the operation. In two rabbits in which the ganglion was removed on one side, the corresponding sympathetic nerves were stimulated forty-five days after the operation. With very strong induction currents there was a moderate constriction of the ear-vessels; no effect upon the pupil. In a third rabbit in which the ganglion was removed on one side, and the sympathetic nerve was resected on the other side, the sympathetic nerves were stimulated seventy-one days after the operation. There was a fair constriction of the blood-vessels on the ganglion side, with a moderate current (300 units, Kronecker's model), while there was no effect at all either on the blood-vessels or on the pupil on the side on which the sympathetic nerve was resected, even when stimulated with very strong currents (3000 units and more). On this side apparently no regeneration took place even after seventy-one days.

We shall not enter into any discussion of the observations we have just recorded. They are not sufficient in number and were gathered only incidentally to our chief studies. The discussion of the latter will be carried out in a subsequent paper.

STUDIES ON THE "PARADOXICAL" PUPIL-DILATATION  
CAUSED BY ADRENALIN. II. — ON THE INFLUENCE  
OF SUBCUTANEOUS INJECTIONS OF ADRENALIN  
UPON THE EYES OF CATS AFTER REMOVAL OF  
THE SUPERIOR CERVICAL GANGLION.

By S. J. MELTZER.

[*From the Hallerianum in Bern.*]

BESIDES its influence upon the pupil, the cervical sympathetic nerve controls also the width of the palpebral fissure, the movements of the nictitant membrane and the position of the bulbus. The influences upon the latter phenomena are indistinct in rabbits, but are outspoken in cats. The studies in the foregoing paper were made on rabbits, and were therefore restricted to the influence of adrenalin upon the pupil. Last summer, while enjoying the unlimited hospitality of the physiological laboratory in Bern (thanks to the kindness of Professor Kronecker), I have made some observations also on the effect of adrenalin upon the eyes of cats. The experiments were made chiefly with subcutaneous injections. The results obtained are, briefly stated, as follows.

Subcutaneous injections of even large doses of adrenalin have no effect upon the eyes of normal cats. When the sympathetic nerve (or more often the vago-sympathetic) was cut on one side, the pupil on the corresponding side became smaller, the palpebral fissure narrower, and the nictitant membrane covered a good part of the eye. (Of the position of the bulbus I was never sure enough, and can therefore make no positive statement with regard to it.) When now one c.c. or more of adrenalin was injected subcutaneously, a retraction of the nictitant membrane very soon followed; this occurred even if the injection was made soon after the operation. But at no time and with no dose of adrenalin could the condition of the pupils or the palpebral fissure be affected by a subcutaneous injection.

However, if the superior cervical ganglion was removed, a subcutaneous injection of adrenalin caused a distinct widening of the pal-

pebral fissure and a complete dilatation of the pupil. This effect could be obtained only when the drug was injected at least 48 hours after the operation (24 hours in rabbits). At least 1.5 c.c. was required to bring out a distinct effect, even in a small and young animal. Older and larger animals required larger doses. The larger the dose, the sooner the phenomena appeared, and the longer they lasted, — the pupil sometimes remaining dilated for six hours and longer. Usually there was immediately after the injection a distinct dilatation of the pupil on the operated side, which, however, disappeared soon again, but reappeared in 15 to 40 minutes, according to the injected dose of adrenalin, to remain then unchanged for hours. The primary dilatation is probably due to the excitement of the animal, or to the pain produced by the injection; since both can, as we now know, bring on the so-called paradoxical dilatation of the pupil. I shall state, however, that by injection of water I could never bring on this primary dilatation of the pupil.

In no instance, even if the pupil was ever so dilated, was the reaction to light lost, or perhaps not even perceptibly affected. (In rabbits, as we have stated above, with the dilatation of the pupil there was also always an impairment or complete loss of the reaction to light.) However, the largest dose of adrenalin which I have ever used subcutaneously on operated cats was 3 c.c., and it is possible that a larger dose might indeed affect also the reaction. (I have avoided larger doses in order not to risk the life of the animal. It is worth mentioning, however, that with the exception perhaps of an occasional diarrhoea, in no case were the cats affected by even a dose of 3 c.c.; while even in a larger rabbit a dose of 2 c.c. would cause a marked paresis and general prostration.)

When the sympathetic nerve was cut on one side, and the ganglion removed on the other side, the contrast between the pupils and the palpebral fissures of the two sides was very striking. The retraction of the nictitant membrane occurred on both sides, but it seemed that on the ganglion side the retraction was more marked and lasted longer than on the sympathetic side. But even on the ganglion side the retraction gave way sooner than the other phenomena.

The described phenomena could be produced at will as long as the animals were kept alive, that is for about six weeks.

I have not been successful with instillations of adrenalin into the eyes of operated cats, on account of the protruding nictitant membrane which would sweep over the entire eye, as soon as the adrenalin



came in contact with it, thus removing the adrenalin. But I must admit that I have not been persistent enough in my attempts.

I may perhaps put on record that in two instances I made an operated cat drink milk to which 6 c.c. of adrenalin had been added. About an hour after the partaking of the milk, the pupil on the ganglion side began to dilate. The dilatation lasted for a few hours, but was never considerable.

In one operated cat which was kept under a moderate ether anæsthesia, and in which there was no perceptible difference between the two pupils, an intravenous injection of 1 c.c. of adrenalin brought on a maximum dilatation of the pupil on the ganglion side which lasted for one hour. At the end of that time the cat was killed, but the dilatation of the pupil persisted for two hours longer.

Like Langendorff,<sup>1</sup> I have also observed that in a deeply etherized cat the pupil on the "ganglion side" becomes greatly dilated, while on the "sympathetic side" the pupil remains unchanged. This occurred only when the deep anæsthesia was carried on and completed under a glass bell, in the same manner as was stated by Langendorff. By this method, however, we have not only anæsthesia by ether, but also asphyxia by carbon dioxide. When, however, in an early stage of the anæsthesia the cat was quickly removed from under the bell and fastened on a board, and the ether administered in free air, the animal was under a fairly good anæsthesia without showing any marked difference between the two pupils.

<sup>1</sup> LANGENDORFF: *Loc. cit.*

## STUDIES ON THE "PARADOXICAL" PUPIL-DILATATION CAUSED BY ADRENALIN. III.—A DISCUSSION OF THE NATURE OF THE PARADOXICAL PUPIL-DILATATION CAUSED BY ADRENALIN.

BY S. J. MELTZER AND CLARA MELTZER AUER.<sup>1</sup>

[From the Rockefeller Institute for Medical Research.]

IN the foregoing reports it has been shown that subcutaneous injections (or instillations) of adrenalin have no effect upon the normal pupil nor upon a pupil whose sympathetic nerve was cut or resected. But if the superior cervical ganglion was removed, adrenalin invariably caused a strong and long-lasting dilatation of the corresponding pupil. The presence of the ganglion then prevents the dilating effect of adrenalin upon the pupil; by its excision something is removed which is normally an obstacle to the dilatation. It has long been known that when the sympathetic nerve is cut, the pupil becomes smaller. The generally accepted interpretation of this phenomenon is that the nerve fibres of the sympathetic are continually carrying mydriatic impulses to the pupil, which are discontinued by section of the nerve. It would seem to be the same logical interpretation to state that by the excision of the ganglion certain normal myotic impulses are removed. But we would then have to assume that the sympathetic nerve carries to the pupil from the central nervous system mydriatic impulses, and that the superior cervical ganglion sends myotic impulses through the postganglionic fibres. In other words, the ganglion generates and sends to the peripheral tissues impulses of its own, which in their character are just opposite to those sent by the central nervous system through the sympathetic nerve fibres.

However, the conception of the cervical sympathetic system as a conveyer of mydriatic impulses to the pupil is more than sixty years old and is deeply rooted. The early and oft expressed claim that the

<sup>1</sup> Research scholar of the Rockefeller Institute for medical research.

superior cervical ganglion sends up impulses of its own had reference only to such impulses as were exactly of the same character as those carried by the sympathetic nerve, — additional mydriatic impulses. When, therefore, facts became known showing the occurrence of the dilatation of the pupil after removal of the ganglion, these facts did not give rise to the logical interpretation that the ganglion might normally send myotic impulses; the phenomena were simply dubbed “paradoxical.”

The term “paradoxical pupil-dilatation” is of very recent origin. It was introduced a few years ago by Langendorff<sup>1</sup> to designate a group of dilatations of the pupil occurring under certain conditions after removal of the superior cervical ganglion. To this group belongs in the first place also the old observation of Budge, that when the sympathetic nerve is cut on one side below the superior ganglion, and on the other side the postganglionic fibres are cut (thus excluding the influence of the ganglion), a day or two after the operation the pupil on the “ganglion side” is a little wider than the one on the sympathetic side. Of the other conditions which favor the appearance of the paradoxical pupil-dilatation we have to mention deep anæsthesia by chloroform, ether, or chloral, asphyxia, and excitement of the animal. If the ganglion were removed on one side, and the sympathetic nerve cut on the other side, these conditions would cause a wide dilatation of the pupil on the “ganglion side,” with no effect upon the pupil on the “sympathetic side.” It was also observed that curare had the same effect.<sup>2</sup>

Besides Langendorff, who studied the phenomena carefully, and who deserves special credit for having directed attention to them, the subject was investigated by many observers before him. Langendorff himself mentions as his forerunners, Budge,<sup>3</sup> Surminsky,<sup>4</sup> Tuwim,<sup>5</sup> and Schiff.<sup>6</sup> Besides these authors, the phenomena were

<sup>1</sup> LANGENDORFF: *Loc. cit.*

<sup>2</sup> It is, however, a question whether in the cases of anæsthesia as well as of curare the paradoxical mydriasis is not due to the concomitant asphyxia. In the above-described observations of one of us upon cats, anæsthesia without asphyxia never brought out the paradoxical pupil-dilatation. The same was observed by H. K. Anderson, who is of the opinion that the effect of anæsthesia “is not direct, but indirect by the anæsthesia it causes.”

<sup>3</sup> BUDGE: *Loc. cit.*

<sup>4</sup> SURMINSKY: *Zeitschrift für rationelle Medicin*, 1869, xxxvi, p. 231.

<sup>5</sup> TUWIM: *Archiv für die gesammte Physiologie*, 1881, xxiv, p. 127.

<sup>6</sup> SCHIFF: *Gesammelte Beiträge*, 1896, iii, p. 107.

carefully studied by Kowalewsky,<sup>1</sup> Braunstein<sup>2</sup> and Roebroek.<sup>3</sup> Since the publication of Langendorff's paper, the subject was studied and discussed by Levinsohn,<sup>4</sup> Lewandowsky<sup>5</sup> and H. K. Anderson.<sup>6</sup>

We should add that the conditions which bring out the paradoxical pupil-dilatation cause also a widening of the palpebral fissure and a retraction of the nictitant membrane.

The paradoxical pupil-dilatation following the injection or instillation of adrenalin, which we have described above, is in most respects similar to the phenomenon observed under the other conditions. It cannot be due, however, to pain or excitement of the animal caused by the injection, since in all cases the dilatation takes place long after the injection; and it cannot be due to asphyxia, since it occurs after the use of such small doses as produce no ill effect whatever upon the animal; and since it is also produced by instillations which exert only a local influence. The effect is apparently due to the specific action of adrenalin upon the concerned tissues. But what is the nature of this action, and which tissues are affected? What is the mechanism of this mydriasis? For the interpretation of the paradoxical pupil-dilatation consequent upon the causes enumerated above (anæsthesia, etc.), there are a number of theories. Budge assumes that the total abolition of the tonus of the dilator pupillæ, which is accomplished by the removal of the ganglion, leads finally also to a relaxation of the sphincter, — a sort of atrophy by disuse. This theory was also accepted by Tuwim, and is at present advocated by Levinsohn. Surminsky assumed that these dilatations are caused by constrictions of the blood-vessels of the iris. Braunstein is of the opinion that the dilatations are caused by a cortical inhibition of the sphincter. Langendorff and also Roebroek assume that, after the removal of the ganglion, the dilator is more or less in a state of contracture kept up by the processes within the degenerating post-ganglionic nerve fibres. Kowalewsky assumes that the ganglion sends

<sup>1</sup> KOWALEWSKY: *Loc. cit.*

<sup>2</sup> BRAUNSTEIN: *Zur Lehre von der Innervation der Pupillenbewegung*, Wiesbaden, 1894.

<sup>3</sup> ROEBROECK: *Het Ganglion Supremum Colli Nervi Sympathici*, Utrecht, van Boekhoven, 1895.

<sup>4</sup> LEVINSOHN: *Klinische Monatsblätter für Augenheilkunde*, 1900, xxxviii, p. 625; *Archiv für Ophthalmologie*, 1902, lv, p. 153.

<sup>5</sup> LEWANDOWSKY: *Sitzungsberichte der Königlich-Preussischen Akademie der Wissenschaften*, 1900, p. 1136; *Archiv für Physiologie*, 1903, p. 367.

<sup>6</sup> H. K. ANDERSON: *Journal of physiology*, 1903, xxx, p. 290.

exciting as well as inhibiting nerve fibres to the dilator. Lewandowsky finally is of the opinion that the dilatation is due to an increased excitability of the dilator muscle owing to the loss of an inhibitory influence exercised by the ganglion over that muscle.

In stating our views as to the nature and course of the paradoxical state in question, we shall restrict our discussion to the pupil-dilatation caused by adrenalin. Neither shall we enter into a general discussion of the availability of the above-quoted theories for our special case. We shall try to state our interpretation of the phenomena observed by us without attempting to controvert the views promulgated by others.

There is, however, one of the older interpretations which seems to have been definitely disposed of by our present-day writers, but which, it seems to us, requires a discussion in our special experience. It is the theory that the pupil-dilatations are caused by a contraction of the blood-vessels. As the constriction of the blood-vessels is the chief characteristic of adrenalin, we have indeed to investigate whether the dilatation of the pupil is not simply due to this constriction.

When Lewandowsky<sup>1</sup> first discovered the brief dilatation of the pupil after an intravenous injection of the suprarenal extract, he already touched upon the question whether it be not due to the vasoconstriction of the vessels of the iris, but dismissed this suggestion by the remark that the dilatation is too great to be caused by the constriction of the vessels. Lewandowsky had apparently in mind the older view of the dilatation of the pupil being caused mechanically by the constriction of the blood-vessels. But with adrenalin there is another side to this question. The constriction of the vessels is so strong as to cause local anæmia or local asphyxia, and the question can be raised indeed whether the pupil-dilatation is not caused indirectly by the local asphyxia. The pupil-dilatation which we observed to occur after administration of adrenalin would thus be simply another variety of paradoxical mydriasis due to asphyxia. This might appear the more plausible since we know that after the removal of the ganglion (or section of the sympathetic), the constriction of the blood-vessels of the ear lasts a long time; and we might justly assume that after their release from the influence of the sympathetic nerve, the blood-vessels of the iris under the influence of adrenalin also remain contracted for a long time.

<sup>1</sup> LEWANDOWSKY: *Loc. cit.*

The facts that the palpebral fissure and nictitant membrane also show a paradoxical behavior, which was formerly adduced as a proof against the blood-vessel theory of the dilatation of the pupil, cannot be used in our case; as the muscles concerned in these phenomena might also be asphyxiated and thus cause the tonic contraction.

There is, however, one fact in our experience which seems to speak against this assumption. For the blood-vessels of the ear we have established that the simple section of the sympathetic is sufficient to prolong the constricting effect of adrenalin, and that the removal of the ganglion does not increase this effect. We are justified in assuming that the same holds good also for the other blood-vessels under the control of the sympathetic, including the vessels of the iris. Nevertheless administration of adrenalin causes dilatation of the pupil only after removal of the ganglion and not after section of the sympathetic nerve. Furthermore, we have often observed a dilatation of the pupil after injection of such a small dose of adrenalin as had no effect upon the blood-vessels of the ear. Finally the dilated pupil after injection of adrenalin persisted for hours after the death of the animal, when there could hardly have been any difference in the state of asphyxia between the tissues of the two eyes. Moreover, we ought also to point out that there is no gain in the assumption that the contraction of the smooth muscle fibres of the eye is due to asphyxia, and not to direct stimulation by the adrenalin. Since we know that this substance causes prompt contraction of the muscular sheath of the blood-vessels, why can we not assume that it can also cause the contraction of the dilator muscle of the pupil?

In presenting now our view of the nature of the paradoxical pupil-dilatation following the administration of adrenalin, we shall state at the outset that we see in this phenomenon an indication indeed, as we have stated at the beginning of our paper, that the ganglion sends normally impulses which prevent the dilatation of the pupil, — impulses which are opposite to those carried by the sympathetic nerve and which are represented, in part at least, by inhibition of the excitability of the dilator muscle. This agrees in general with the views of Kowalewsky and of Lewandowsky. We further agree with Lewandowsky and Anderson that the paradoxical phenomena are due, in part at least, to an increased excitability of the contractile tissues which have been freed from the influence of the ganglion. We finally also agree with the views of Lewandowsky and Langley that the suprarenal extract causes dilatation of the pupil and the

other smooth muscles of the eye by directly stimulating the contractile tissues. In order to state our own view more explicitly, however, we have to go a little further.

It is now well established that the dilatation of the pupil is accomplished by the contraction of a muscle, the dilatator pupillæ, just as the constriction of the pupil is accomplished by the contraction of the constrictor pupillæ. Both muscles are exact antagonists, just as are such muscles as the extensors and flexors of an extremity; or as the rectus externus and internus of an eye. The constriction and dilatation of the pupil change with great rapidity, and the rapid play goes on nearly continually. Could such a rapid play ever take place if during the contraction of one muscle its antagonist should still be contracted and would simply have to be overpowered by sheer force? This seems hardly to require any discussion. Nobody claims it. The one antagonist is relaxed in proportion as the other contracts. What causes the relaxation? Is it simply a withdrawal of the impulse for contraction? We deal here with smooth muscle fibres whose contraction when once started would last a good many seconds, while the changes in the width of the pupil take place within a fraction of a second. The conclusion is unavoidable that the relaxation is an active one, — an inhibition of the contraction, not only of a tonus, as is often stated, but of an active contraction. And the further conclusion is, therefore, unavoidable that with each contraction of the dilator a simultaneous proportionate inhibition of the constrictor takes place, and, conversely, with each contraction of the constrictor a simultaneous inhibition of the dilator takes place.<sup>1</sup>

<sup>1</sup> I wish to append here the following remark: In my paper *Das Schluckcentrum, seine Irradiationen*, etc. (DU BOIS REYMOND'S *Archiv für Physiologie*, 1883, p. 209), I stated that in order to execute proper locomotion the nerve which controls flexion ought also to carry inhibitory fibres for the extension of an extremity. I have then called attention to the fact that such a proper antagonistic arrangement is to be found in the nervous mechanism of the respiratory function; for instance, the stimulation of the superior laryngeal, the second branch of the trigeminus and the central end of the splanchnic nerves, causes a contraction of the expiratory, and, simultaneously, an inhibition of the inspiratory muscles. I have also pointed out that from the "expiratory centres" (CHRISTIANI) active expiration and inhibition of inspiration can be produced; *i. e.* "that the centre for inhibition of inspiration is anatomically associated with that for active expiration and not with that for active inspiration." It was not until a good many years later that SHERRINGTON showed that stimulation of the central end of the nerve for the flexors (hamstring muscles) causes an inhibition of the extensor cruris; and that SHERRINGTON and H. E. HERING demonstrated that the same cortical area

In this connection the experiments of E. Waymouth Reid<sup>1</sup> on the electrical currents of the iris during dilatation and constriction are of considerable interest. We know now that during the contraction of a muscle the electrical current is of a negative, and during its inhibition of a positive variation (Gaskell). Now Reid connected one pair of electrodes with the constrictor, and another pair with the dilator of the pupil, and observed the character of the currents which appeared during constriction and dilatation. When dilatation of the pupil was caused by stimulating the peripheral end of the sympathetic nerve, it was found that the current of the dilator showed a negative variation, while that of the constrictor showed a positive variation. This shows that the impulses sent through the sympathetic to the pupil are of two kinds: excitation to the dilator and inhibition to the sphincter. The reverse occurred when a constriction was caused by stimulating intracranially the oculo-motor nerve.

We have said above that the impulses sent by the ganglion are just the opposite to those carried by the sympathetic nerve. We can now say more precisely that the impulses of the ganglion are: inhibition of the dilator and excitation of the constrictor. As is usual with all antagonistic nerve fibres when they are stimulated simultaneously with the same strength, a resultant follows showing considerable predominance of the effects of one of the nerves. (Vagus

from which one group of muscles can be made to contract contains also the centre for inhibition of the antagonistic group of muscles. V. BASCH and EHRMAN, and FELLNER and v. ZEISSL have stated that the nerve trunk which contains the fibres inhibiting the circular muscle fibres of the small or large intestine, or the constrictor vesicæ, carries also the nerve fibres which cause contraction of the (antagonistic) longitudinal muscle fibres of the intestine or the detrusor vesicæ, and *vice versa*. They called this nervous arrangement of the organs "crossed innervation." SHERRINGTON designated the correlated phenomena which he observed on skeletal muscles "reciprocal innervation." In my second paper on inhibition (The Rôle of Inhibition, etc., Medical Record, 1902, p. 881), in which I have shown that the inhibition of one group during the activity of the antagonistic group of muscles, smooth or striated, is a wide-spread phenomenon through many of the mechanisms of animal life, I have termed this correlation: The Law of Crossed Innervation. In a recent paper, however (Archiv für Verdauungskrankheiten, 1903, ix, p. 450), in which I have dealt extensively with the same subject, I have given up, for reasons stated there, the term Crossed Innervation and designated the relations under discussion as The Law of Contrary Innervation. The inhibition of the sphincter pupillæ during an excitation of the dilator, or the inhibition of the dilator during a contraction of the pupil, is a good illustration of the Law of Contrary Innervation. S. J. M.

<sup>1</sup> E. WAYMOUTH REID: Journal of physiology, 1895, xvii, p. 438.



and augmentor, sympathetic nerve and chorda, erigentes and hypogastrici, etc.) Of the cells of the ganglia some serve for the transmission of impulses from the central nervous system, brought to the ganglia through the sympathetic nerve fibres, and some generate new impulses. When the ganglion is stimulated, the effect upon those elements which carry the impulses from the central nervous system predominate. Hence stimulation of the ganglion will always cause a more or less distinct dilatation of the pupil (H. K. Anderson). It is possible also that the impulses sent by the ganglion are normally weaker than those sent by the central nervous system. When, however, by cutting the sympathetic nerves, the impulses carried by these fibres become eliminated, then the impulses of the ganglia come to the front. The constriction of the pupil after section of the sympathetic nerve is, perhaps, due not only to the elimination of the tonic impulses from the central nervous system, but also to the new activity of the previously overpowered antagonistic impulses of the ganglion. When, after a previous section of the sympathetic nerve, the ganglion is removed by excision (or evulsion), the very act of the excision<sup>1</sup> will, in the first place, act as an additional stimulus upon the postganglionic fibres which normally carry the subdued ganglionic impulses. The first effect of the removal will, therefore, sometimes be an increase in the constriction of the corresponding pupil; at all events it will not diminish the constriction. Since after removal of the ganglion there are no opposing impulses to overcome the stimulating effect of the section, or evulsion, this effect will wear off only slowly, after hours or even days. Gradually, however, the stimulating effect of the cut or tear disappears, and the pupil is now released from the hold of the ganglion. The first visible effect is a slight dilatation of the pupil again. But among other usually not noticeable effects is the increased excitability of the dilator muscle (after being freed from the inhibitory impulses of the ganglion), and the diminished readiness of the constrictor (after being deprived of the continually exciting impulses).

We now know that the suprarenal extract can cause a constriction as well as an inhibition of smooth muscle fibres. It causes constriction of the blood-vessels, contraction of the erector muscles of the hair, of the uterus of the rabbit, etc., and it causes inhibition of the

<sup>1</sup> Any cut can be felt for many hours and days; at the cut end of a nerve a stimulation is apparently going on for some time, even though its objective effect cannot be demonstrated in all cases.

stomach, intestines, bladder, etc. Our assumption with regard to the pupil is that adrenalin affects it by exciting the dilator and inhibiting the constrictor. When the normal impulses of the superior cervical ganglia are present and active, they antagonize these effects. Subcutaneous injections or instillations which carry slowly and only minute doses of the suprarenal extract to the muscles of the iris, are easily overcome by the antagonistic impulses emanating from the ganglion, and even when injected intravenously the effect is very brief, since the effective dose is soon diminished and its balance is overpowered by the impulses from the ganglion. When, however, the ganglion and its antagonistic impulses are removed, and the stimulating effect of the cutting has vanished, or has been greatly diminished, the suprarenal extract meets with no antagonism, and hence the prolonged effect of an intravenous injection of adrenalin upon the blood-vessels and pupil, and the pupil-dilating effect of a subcutaneous injection or an instillation of even a comparatively small dose of it.

Furthermore, we are of the opinion that the long-lasting effect in these cases is due also to the absence of an antagonistic factor to cut it short, and it seems to us not unreasonable to assume that the continuation of the effect for some time after death is due also to the absence of a factor to remove it.

To sum up our view in a few words: we believe that the normal effect of the superior cervical ganglion is to inhibit the dilator and to stimulate the constrictor, and the effect of suprarenal extract is just the reverse of it, *i. e.* to excite the dilator and inhibit the constrictor. The extract can, therefore, show its proper effect only after the antagonistic activity of the ganglion is removed.

The "paradoxical" opening of the lids after subcutaneous injection of adrenalin can be explained on the same lines as that of the paradoxical pupil-dilatation; but we shall not enter here into a detailed discussion of it.

Neither shall we enter at present into a discussion of the obvious applicability of our interpretation also to the paradoxical pupil-dilatation caused by other factors (anæsthesia, etc.). We offered our interpretation of the phenomena observed by us as a working hypothesis. Its general applicability can wait until it is tested by more work.

The phenomena in the eye we have found while testing the hypothesis which we offered in explanation of the observations we made

on the ear-vessels. We found that on intravenous injection of adrenalin the blood-vessels of the ear on the side on which the sympathetic and cervical nerves were cut remained constricted incomparably longer than on the normal side. We explained this by the assumption that the section of the nerves excluded the central vasodilating effect, assuming further that the suprarenal extract within the blood is soon reduced to the value of a weak stimulus which favors dilatation. In pursuance of this hypothesis, we found further that subcutaneous injection of moderate doses of adrenalin can indeed cause a dilatation of the ear-vessels of a normal animal, but that the same dose will invariably cause a long-lasting constriction of the ear-vessels when the sympathetic and cervical nerves had been previously cut. For the pupil we found that a subcutaneous injection has no effect on the normal, nor is the effect improved when the sympathetic nerves are cut. But subcutaneous injections, as well as instillations, have a marvellous effect when the superior cervical ganglion is removed. In other words, subcutaneous injection has practically no effect upon either vasoconstrictors or pupil; intravenous injection affects the pupil for a fraction of a minute, and the vasoconstrictors for four or five minutes. But after exclusion of the central influences, the constriction (of ear-vessels) lasts an hour and longer, and after removal of the ganglion, the dilatation of the pupil lasts for hours, whether the adrenalin was administered intravenously or subcutaneously. It is the central nervous system which interferes with the vasoconstriction, and it is the ganglion which interferes with the pupil-dilatation. Our first hypothesis reads that the central nervous system interferes with the vasoconstriction by means of the usual antagonism of the latter, *i. e.* by means of vasodilatation; our present hypothesis reads that the ganglion interferes with the pupil-dilatation by the usual antagonism to the latter, *i. e.* by means of the inhibition of the dilator and excitation of the constrictor.

We should point out the fact that the interference of the central nervous system concerns an organ which has only one set of muscle fibres — the constricting muscle fibres of the blood-vessels; while the interference of the ganglion concerns organs which have antagonistic sets of muscle fibres — the constrictor and dilator of the pupil (and also the muscles increasing and decreasing the width of the palpebral fissure). In our experience the interference with the nictitant membrane came from the central nervous system, — the membrane readily

retracted on injection of adrenalin after section of the sympathetic nerve. According to the emphatic statement of Lewandowsky, there is no antagonistic muscle to the membrane. We should mention here that in our studies on the effect of the cervical sympathetic system on inflammation produced in the ear, we found that the course is much more unfavorably affected by the section of the sympathetic nerve than by the excision of the superior cervical ganglion. We had to explain these surprising conditions also by the assumption that the metabolic influences of the ganglion are exactly the reverse of those exerted by the sympathetic nerves.

We should once more call attention to the fact that our experiments with the subcutaneous injections of adrenalin throw sufficient light also upon another question of general character, namely, whether the suprarenal extract is destroyed through oxidation by the blood and tissues. The short duration of the rise of blood-pressure after an intravenous injection of the extract was explained by Szymonowicz<sup>1</sup> and Cybulski and especially by Langlois<sup>2</sup> and his co-workers<sup>3</sup> to be due to a rapid oxidation of the extract by the blood. The failure to obtain the effect upon the pupils as well as upon the blood-pressure by subcutaneous injections was also explained through the oxidation of the extract by the tissue (Lewandowsky, Boruttau). Very recently Embden and v. Fürth<sup>4</sup> have in a number of experiments mixed the extract (suprarenin, v. Fürth) with blood and various tissues and found that the effect of the extract did not suffer even by prolonged contact. Against this evidence it could, however, be claimed that living tissues might act differently upon the extract than the dead substances which were used by these authors.

Our many experiments which have shown that the effect upon the blood-vessels as well as upon the pupil can be considerably prolonged if only the sympathetic nerves were cut or the superior cervical ganglion removed, demonstrate sufficiently that the extreme brevity of the effect, or the failure to produce any effect, cannot be due to a rapid destruction of the extract through oxidation by the tissues. However a most conclusive demonstration how little the living tissues affect the activity of the extract is to be found in our

<sup>1</sup> SZYMONOWICZ : *Archiv für die gesammte Physiologie*, 1896, lxiv, p. 143.

<sup>2</sup> LANGLOIS : *Comptes rendus de la société de biologie*, 1897, xlix, p. 524.

<sup>3</sup> ATHANASIVS and LANGLOIS : *Ibid.*, p. 575.

<sup>4</sup> EMBDEN and v. FÜRTH : *Hofmeister's Beiträge für chemische Physiologie und Pathologie*, 1903, iv, p. 421.

experiments with subcutaneous and intramuscular injections of adrenalin into an extremity beneath a ligature. Here the adrenalin remained in intimate contact with the tissues for two hours and longer without losing any of its effects. In fact, the effects set in more promptly and rapidly than after a simple subcutaneous injection.

These experiments seem to speak also against the supposition made by Embden and v. Fürth that the suprarenal extract might be partly destroyed by the alkalinity of the blood. We are not aware that the alkalinity of the tissues is markedly less than that of the blood. Nevertheless, the efficiency of the extract, as our experiments show, did not seem to suffer by a prolonged intimate contact with the living tissues.

Our experiments teach also, we believe, an important pharmacodynamic lesson. The suprarenal extract or adrenalin is now a much-employed drug. The general knowledge of its activity was derived from studies made upon normal animals. From these studies it was learned that subcutaneous injections have hardly any influence upon blood-pressure and have no influence upon the pupil. We have shown that when the sympathetic is cut or the superior cervical ganglion is removed, such injections of adrenalin exert a very prompt influence indeed and one of much longer duration than in normal animals. Now our knowledge of the effects of all drugs and animal extracts is derived from studies upon normal animals. Is that sufficient? What is their effect upon animals which are deprived of certain nervous or other influences? Might we not find in many, if not in most, of the cases, influences of an entirely different character? This is the more important, since we are interested in drugs chiefly on account of their possible influence upon the animal organism when the latter is in a pathological condition.

## RHYTHMS OF SUSCEPTIBILITY AND OF CARBON DIOXIDE PRODUCTION IN CLEAVAGE.

By E. P. LYON.

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TWO years ago I described<sup>1</sup> certain phenomena observed in the segmentation of the echinoderm egg which indicated that the successive stages of each cell division are accompanied by varying susceptibility to poisonous agents. It was shown that the egg is especially susceptible to potassium cyanide about ten or fifteen minutes after fertilization. Gradually the resistance rises until the first cleavage. Then comes a second susceptible period, followed by one of resistance. Apparently this rhythm of susceptibility and resistance accompanies still further the rhythm of morphological changes of cleavage. Perhaps it may prove to be a constant phenomenon of cell division and give some insight into delicate physiological processes of which the visible changes so often studied must be only a rough suggestion.

This becomes more likely when one recalls the fact, also recorded in my former paper, that rhythms of susceptibility and resistance to lack of oxygen, no matter how produced, may be demonstrated. The periods of susceptibility were found to be the same for eggs exposed to a hydrogen stream as for those exposed to potassium cyanide.

It seemed worth while, therefore, during my stay at Woods Hole last summer to test the susceptibility of the egg during cleavage to other agents. Heat and cold particularly gave marked results.

**Effects of heat.**—In these experiments several similar dishes were prepared by careful cleaning and sterilization. Equal quantities of unfertilized *Arbacia* eggs were placed in the dishes. These dishes were kept at the same temperature. To dish No. 1 was added a known quantity of sperm. Ten minutes later (or after any other desirable interval) the eggs in dish No. 2 were fertilized by the same

<sup>1</sup> This journal, 1902, vii, p. 56.

amount of sperm. Again after an interval, dish No. 3. This continued until six to ten different lots had been fertilized at as many different times. Thus at a time when the eggs in dish No. 1 might be in the two-cell stage, perhaps dish No. 3 would be approaching cleavage and dish No. 10 be only just fertilized.

Meanwhile in a large bath of warm water an equal number of dishes, containing equal amounts of sea-water and carefully brought to even temperatures, had been provided. The fertilized eggs were quickly transferred to the similarly numbered dishes of warm sea-water. After proper intervals, equal quantities were removed from each of the warm dishes, returned to numbered dishes of sea-water at room temperature, and left to develop. Later the percentage of larvæ produced or the percentage of cleavage was ascertained for each sample, or more general statements of the degrees of development recorded. The results are best expressed in tabular form.

EXPERIMENT I.

No. of minutes elapsed after fertilization before eggs exposed to heat . . . . .	7	15	23	30	40	48
Per cent plutei from eggs exposed to 32° C. for 10 minutes . . . . .	85	60	65	30	30	26
Per cent plutei from eggs exposed to 32° C. for 16 minutes . . . . .	67	58	67	26	36	18

EXPERIMENT II.

No. of minutes elapsed after fertilization before eggs exposed to heat . . . . .	5	10	20	30	40	50
Per cent plutei from eggs exposed to 33° for 10 minutes . . . . .	19	70	54	15	5	4—

EXPERIMENT III.

No. of minutes elapsed after fertilization before eggs exposed to heat . . . . .	2	10	20	30	40	50
Per cent plutei from eggs exposed to 35° for 4 minutes . . . . .	2.5	4	5	1	0.1	0

These results make it plain that the eggs at the period just before division are more easily injured by the treatment to which they were subjected than at earlier stages. Immediately after the entrance of the sperm the egg is more susceptible than a few minutes later. The period of greatest resistance seems to correspond fairly well with that

of greatest susceptibility to potassium cyanide. Indeed, the curve of heat injury would seem to be the reverse of that of potassium cyanide or lack of oxygen injury. That the effects described are recurrent or rhythmical is indicated by the following experiments.

## EXPERIMENT IV.

In this culture the first cleavage took place in the interval between sixty and seventy minutes after addition of sperm. In other words, at the time of heating, the sixty-minute specimen contained very few two-celled forms, while the seventy-minute specimen consisted chiefly of two-celled stages.

No. of minutes elapsed after fertilization before eggs exposed to heat . . . . .	5	40	50	60	70
Per cent of plutei developed after 7 minutes at 34°	24	2	1	0	20

## EXPERIMENT V.

In this experiment, an observation made fifty-five minutes after fertilization showed ten per cent in two-celled stage, while sixty-eight minutes after fertilization practically all had passed the first cleavage.

No. of minutes elapsed after fertilization before eggs exposed to heat . . . . .	3	33	47	55	68	73	78	88
Per cent plutei after exposure to 35° for 5 minutes . . . . .	12	37	1	14	90	25	10	5
Per cent plutei after exposure to 35° for 9 minutes . . . . .	1	20	0	2	10	16	0	0

The fact that 10 per cent had divided in the fifty-five-minute sample before it was exposed to heat explains, perhaps, why a larger number of plutei formed here than in the forty-seven-minute specimen. *All the experiments indicate that the cell (in arabacia) is especially sensitive to heat just before division and resistant just after division.* There is a definite rhythm of susceptibility to heat.

**Effects of cold.**—The method here was essentially the same. Several dishes of sea-water were kept surrounded for some hours with crushed ice, in a large vessel. The whole was kept in a refrigerator. To these dishes the eggs, fertilized at varying intervals previously, were transferred in equal quantities. A number of hours later samples from each dish were warmed to room temperature and allowed to develop. Observations made at intervals revealed the amounts of cleavage in the different samples, or the percentages of larvæ produced.



EXPERIMENT A.

Part of the eggs were at 0° for 16 hours, at the end of which time no change, visible with a low power, had occurred in any of them. They were then allowed to come to room temperature. Another part remained at 0° for thirty-nine hours, and then exhibited no change on examination with a low power. These were then allowed to develop at room temperature.

No. of minutes elapsed after fertilization before eggs placed in ice . . . . .	5	10	15	20	30	45
Observations 1½ hours after removal of 16-hour specimens from ice . . . . .	No cleavage	Hundreds in 2 cell	No cleavage	No cleavage	50% in 2 cell	10% in 2 cell
Observations 2¼ hours after removal of 16-hour specimens from ice . . . . .	Much cleavage	Nearly 100% in 2-8 cell	Some 2-4 cell	Some 2-4 cell	Very numerous 4-8 cell	Numerous 4-8 cell (less than 30-min. spec.)
Observations of development of 39-hour specimens	No larvæ	Practically no larvæ	No larvæ	Practically no larvæ	Numerous larvæ	A few larvæ

It was noticed that while no change could be detected in the eggs as long as they were on ice, rapid division of the nucleus without division of cytoplasm might take place as soon as they were warmed. In many cases degenerative changes were soon apparent. In other cases there might be little difference between the cultures during cleavage, but great differences later on, the injury caused by the cold only manifesting itself in advanced stages of development. This is shown by the next experiment, in which some of the eggs were kept in ice for eighteen hours; others for thirty-six hours.

EXPERIMENT B.

No. of minutes elapsed after fertilization before eggs placed on ice . . . . .	3	10	15	30	40	50
Per cent of eggs in cleavage in 3 hours, after 18 hours on ice . . . . .	60	55	50	70	65	60
No. of swimming larvæ next day, 18-hour specimen . .	Few	Few (less than 3 min.)	Few (less than 10 min.)	Very fine lot	Many	Few and imperfect
No. of swimmers from 36-hour specimen . . . . .	Two or three hundred	About two dozen	Almost none	Several hundred	Few	Very few

In the experiments given, it is noticeable that those eggs which were placed on ice about ten to twenty minutes after fertilization gave the smallest amount of cleavage and the fewest larvæ. Experiment C illustrates the same fact and also that rate of return to room temperature affects the results. The seventeen-hour specimen was allowed to warm without precautions, while the twenty-five-hour sample was very slowly warmed. The latter, in spite of the longer exposure to cold, gave the larger number of larvæ.

## EXPERIMENT C.

No. of minutes elapsed after fertilization before eggs were exposed to ice . . . . .	5	15	20	40
Per cent of larvæ resulting from sample exposed to ice 17 hours . . . . .	5	0	0	100
Per cent of larvæ resulting from sample exposed to ice 25 hours . . . . .	Very numerous	1--	15	100
Per cent of larvæ resulting from sample exposed to ice 50 hours . . . . .	A few	0	0	A few

I performed no experiments with cold involving the interval between first and second cleavage, and therefore have no data for a rhythm of susceptibility and resistance in eggs exposed to low temperatures. To my mind, however, such a rhythm is probable.

*In general, it appears that the egg is most sensitive to cold at about the same stage when it is susceptible to lack of oxygen.* But the most resistant period for cold is earlier than that for lack of oxygen, if one may judge from the data at hand. It will be noticed, however, that these experiments are not accompanied by such careful numerical results as those on heat. This was unavoidable at the time, and therefore the effects of cold will be made a subject for further study later.

## EXPERIMENT D.

No. of minutes elapsed after fertilization, before eggs were exposed to low temperature . . . . .	5	10	15	20	33	50
Per cent of plutei produced from eggs kept at 0° for 26 hours . . . . .	12	13	3--	3--	14	12
Per cent of plutei produced from eggs kept at 0° for 42 hours . . . . .	20	13 <sup>1</sup>	5 <sup>2</sup>	5 <sup>2</sup>	14 <sup>1</sup>	

<sup>1</sup> Large and strong.
<sup>2</sup> All weak and small.

The one experiment in which careful counts of plutei produced from cooled eggs were made bears out, however, the conclusion stated above. It also probably illustrates the effects of gradual *versus* sudden warming, although my notes are not sufficiently full on this point (see Experiment D).

#### PRODUCTION OF CARBON DIOXIDE.

That the metabolism of the egg at different stages of karyokinesis varies greatly is presumable from morphological studies. As a measure of katabolic processes in protoplasm the carbon dioxide production is often taken. I attempted to do this in the segmenting egg and have published the results in the form of a preliminary.<sup>1</sup> It may be stated here that the apparent conclusion was that *carbon dioxide production in the egg is not uniform throughout the whole series of morphological changes of cell division, but rather reaches a maximum at the time when the cytoplasm is actively dividing.* Furthermore, it seemed that at the time when oxygen is most necessary and presumably is being used in largest amount (as indicated by susceptibility to lack of oxygen and to potassium cyanide) carbon dioxide is produced in least amount. But I have used the words "apparent" and "seemed" to indicate that these statements may need revision in the light of later and more accurate investigations. If the conclusion above expressed should justify itself, it would indicate that oxygen is chiefly used in the egg for synthesis rather than for combustion, and that the larger part of the carbon dioxide comes from splitting processes. One would also infer that the energy for cell division comes from fermentative rather than oxidative processes.

Regarding the significance of these rhythms, I am even less inclined to speculate than on the occasion of publication of my former paper. I hope to study them in cells more favorable than the pigmented egg of *arbacia* for seeing interior conditions. I wish also to measure more accurately the carbon dioxide production during cleavage before discussing the theoretical bearings of the experiments. I am not making these remarks, however, with the end in view of monopolizing this interesting field of research. I should be glad, indeed, to have others undertake similar studies in the same or other forms.

<sup>1</sup> LYON: Science, n. s., 1904, xix, p. 350.

## SUMMARY.

1. The fertilized egg of *arbacia*, if exposed to temperatures of  $32^{\circ}$  to  $36^{\circ}$  for a few minutes and then allowed to develop at room temperature, shows a marked difference, depending on the time after fertilization that the heating takes place. It is especially sensitive to heat just before cleavage. It is most resistant ten to twenty minutes after fertilization. Just after the first cleavage it is again resistant to heat, but becomes susceptible again just before second cleavage. There is, therefore, a rhythm of susceptibility and resistance.

2. The effect of exposing *arbacia* eggs to low temperatures ( $2^{\circ}$  to  $0^{\circ}$  C.) for several hours and then allowing development varies greatly, being also dependent on the length of time after fertilization that the lowering of temperature takes place. The susceptible period for cold is quite different from that for heat, being, in fact, more nearly or perhaps exactly the same as the resistant period for heat (ten to fifteen minutes after fertilization). This is also the stage susceptible to lack of oxygen. The resistant period for cold seems to come some minutes before the first cleavage and thus does not coincide with the stage most susceptible to heat.

3. The production of carbon dioxide in the segmenting *arbacia* egg probably runs in rhythms, the greatest amount being produced at the time of active cytoplasmic division.

# CHANGES IN HEART-RATE, BLOOD-PRESSURE, AND DURATION OF SYSTOLE RESULTING FROM BICYCLING.

BY WILBUR P. BOWEN.

[From the *Physiological Laboratory of the University of Michigan.*]

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## I. INTRODUCTION.

IT is well known that muscular work has a marked influence on the action of the heart and the arterial tension. Under certain conditions this influence is normal, healthful, and invigorating; under other conditions, which we have not yet learned to distinguish clearly, it may result in serious injury, — permanent dilatation, valvular incompetence, and even rupture of the heart wall having been known to follow from violent exertion. It is, therefore, of the utmost importance to physical education that extensive and careful studies should be made with a view to discovering more fully how the circulatory system is affected by muscular work of different kinds and degrees of intensity under various conditions. Furthermore, since we do not know at what stage of muscular work these various influences are at a maximum, it is not sufficient to note the after-effects, but the changes in heart-rate, blood-pressure, etc., must be followed from minute to minute as long as the influence of the work continues.

In a previous research the writer made such a study of the changes in pulse-rate accompanying and following several kinds of work.<sup>1</sup> Since forms of work in which the pulse can be recorded continuously

<sup>1</sup> BOWEN: Contributions to medical research. University of Michigan, 1903, p. 462.

are also favorable for the determination of blood-pressure, this research has been extended with the object of ascertaining the relative intensity of the changes in pulse-rate and blood-pressure which occur during moderate exercises of speed, as exemplified by bicycling. A number of the experiments were also utilized for further study of some changes in duration of systole which the writer had observed in previous work. The experiments were made in the Physiological Laboratory of the University of Michigan between January 1 and August 15, 1903. A study of the changes in quantity of respired air and in the output of carbon dioxide during work, made in company with Mr. G. O. Higley of the Department of Chemistry, will be published soon.

## II. PREVIOUS WORK.

In the decade between 1890 and 1900 there were several extensive studies of the after-effects of muscular work upon the pulse,<sup>1</sup> but studies of the heart-rate during the progress of muscular work date from 1900. During that year there were three researches upon the subject: one in Vienna by Grünbaum and Amson;<sup>2</sup> one in Dorpat, Russia, by Dehio;<sup>3</sup> and one in Ann Arbor by the present writer. Grünbaum and Amson counted the subject's pulse with the aid of a stop-watch during work on several gymnastic machines and a bicycle; Dehio counted the pulse while the subject, lying horizontally on his back, lifted a weight attached to the foot; in my work, a graphic record of the pulse was taken during work upon a bicycle and a foot-power lathe, and also while tapping a Morse key.

Grünbaum and Amson looked for the general course of the changes in pulse-rate during and after the work, and tried to correlate the extent of these changes with the amount of external work done. Dehio studied the difference in the effects of work upon persons of different ages, and found a greater acceleration of the pulse in the younger subjects. I studied the same points covered by Grünbaum and Amson, and also gave special attention to the latent period.

The study of blood-pressure during work was made first upon the horse: originally by Marey,<sup>4</sup> and afterward by Kaufmann,<sup>5</sup> and by

<sup>1</sup> See my former article for bibliography of this work.

<sup>2</sup> GRÜNBAUM and AMSON: *Deutsches Archiv für klinische Medicin*, 1901, lxxi, p. 539.

<sup>3</sup> DEHIO: *St. Petersburger medicinische Wochenschrift*, 1901, p. 79.

<sup>4</sup> MAREY: *Physiologie médicale du sang*, Paris, 1863.

<sup>5</sup> KAUFMANN: *Archives de physiologie*, 1892, p. 279.

Zuntz and Hagermann.<sup>1</sup> These writers agree that in the horse the arterial pressure falls as soon as the animal begins to walk or trot. Tangl and Zuntz<sup>2</sup> found that in case of the dog a rise of pressure occurs when work begins.

The after-effect of muscular work upon blood-pressure in man, has been studied by a great many observers, among whom may be mentioned Hill,<sup>3</sup> Edgecumbe and Bain,<sup>4</sup> Maximowitch and Rieder,<sup>5</sup> Kornfeld,<sup>6</sup> and McCurdy. All of these writers report an increased arterial pressure immediately after the cessation of work with a return to normal after a variable time depending upon the severity and the duration of the work.

McCurdy<sup>7</sup> was the first to call attention to the necessity of determining the pressure during the progress of the work, and he studied the pressure in man during maximal effort in lifting. Masing<sup>8</sup> has studied the changes in blood-pressure while the subject worked upon the apparatus used by Dehio in studying pulse-rate. Masing found that although work of the type used causes a faster pulse-rate in the young, it causes a higher blood-pressure in older persons. Moritz<sup>9</sup> made a similar study, comparing the effects of work in sound men and men suffering from heart diseases.

The duration of systole has been studied by Thurston,<sup>10</sup> Edgren,<sup>11</sup> Hürthle,<sup>12</sup> Chapman,<sup>13</sup> and others, using graphic records of the pulse as the basis of the determinations. Muscular work was sometimes used by these writers as a means of increasing the pulse-rate, but none of them determined the duration of systole during the work. Chapman assumed that the duration of systole always bears a certain numerical relation to the duration of the entire cycle, and constructed a table based upon this assumption, showing the length of systole for

<sup>1</sup> ZUNTZ and HAGERMANN: *Stoffwechsel des Pferdes*, Berlin, 1898, p. 382.

<sup>2</sup> TANGL and ZUNTZ: *Archiv für die gesammte Physiologie*, 1898, lxx, p. 544.

<sup>3</sup> HILL: Schäfer's text-book of physiology, 1900, ii, p. 80.

<sup>4</sup> EDGECOMBE and BAIN: *Journal of physiology*, 1899, xxiv, p. 48.

<sup>5</sup> MAXIMOWITCH and RIEDER: *Deutsches Archiv für klinische Medicin*, 1890, xlv, p. 329.

<sup>6</sup> KORNFELD: *Wiener medicinische Blätter*, 1899, p. 631.

<sup>7</sup> MCCURDY: *This journal*, 1901, v, p. 95.

<sup>8</sup> MASING: *Deutsches Archiv für klinische Medicin*, 1903, lxxiv, p. 253.

<sup>9</sup> MORITZ: *Deutsches Archiv für klinische Medicin*, 1903, lxxvii, p. 339.

<sup>10</sup> THURSTON: *Journal of anatomy and physiology*, 1876, x, p. 494.

<sup>11</sup> EDGREN: *Skandinavisches Archiv für Physiologie*, 1889, i, p. 67.

<sup>12</sup> HÜRTHLE: *Archiv für die gesammte Physiologie*, 1891, xlix, p. 66.

<sup>13</sup> CHAPMAN: *British medical journal*, 1894, i, p. 511.

all pulse-rates from 45 to 200. According to this table, the diastolic part of the cycle shortens faster than the systolic, as the pulse-rate quickens, but the systole is always the shorter, even with the highest pulse-rates. This is contradicted by results obtained previously by Thurston, and later by Zuntz and Schumburg,<sup>1</sup> both finding a time when diastole becomes shorter than systole. In my experiments the diastole is uniformly shorter than systole during work when the pulse-rate exceeds 135 per minute (see Fig. 4).

### III. METHODS.

In order to study the effects of bicycling in the laboratory, a bicycle frame from which the wheels have been removed is fastened firmly in its usual position, and the rear sprocket is mounted on a shaft carrying a heavy fly-wheel. The fly-wheel used in this instance weighs one hundred fifty pounds and is geared to run slightly faster than the crank shaft. The momentum of the fly-wheel carries the pedals past the dead point, so that the work done upon the machine is like riding a wheel on a smooth road. A strip of leather belting drawn against the polished rim of the fly-wheel provides a variable resistance, the tension of the belting being changed at will by changing the amount of weight suspended from its free end. The amount of resistance is greatest at the moment of starting, but during continuous movement this method of applying friction gives a resistance that is practically uniform. The resistance at the pedals which corresponds to any given tension of the belting can be determined approximately by means of a light lever and a spring balance. The lever is fastened to one crank, and the balance fastened to the lever at some distance from the axis which bears a simple relation to the length of the crank. The crank is now turned by pulling upon the balance at right angles to the lever, and the balance is read while the movement is being made. From the figures thus obtained the resistance at the pedals is readily computed.

The pulse is recorded from the carotid artery by means of two tambours connected by a rubber tube of four millimetres bore. The receiving tambour, made from a tin box-cover, is five centimetres broad, and is without the usual membrane. The open side of the tambour is placed against the skin of the neck over the artery and held in place by a U-shaped wire spring, one end of which is attached

<sup>1</sup> ZUNTZ and SCHUMBURG: *Physiologie des Marsches*, Berlin, 1901.



to the tambour by a ball and socket joint, and the other end inserted into a rounded block which rests against the opposite side of the neck. The receiving tambour is fifteen millimetres in diameter, and has a light rubber membrane which transmits its movements to the recording surface by a light celluloid lever.

The recording surface consists of a strip of smoked paper 12.5 centimetres wide, and from four to five metres long, made into a loop which passes around two drums, one of which is actuated by clock-work. Above the pulse-curve a pneumograph records the respiratory movements. Below are the records made by three electromagnetic signals: one of these, connected with a fork or pendulum, records the time; another, the revolutions of the bicycle crank; the third, in circuit with a hand-key, is used to indicate when determinations of blood-pressure are made. Fig. 1 shows the general character of the records.

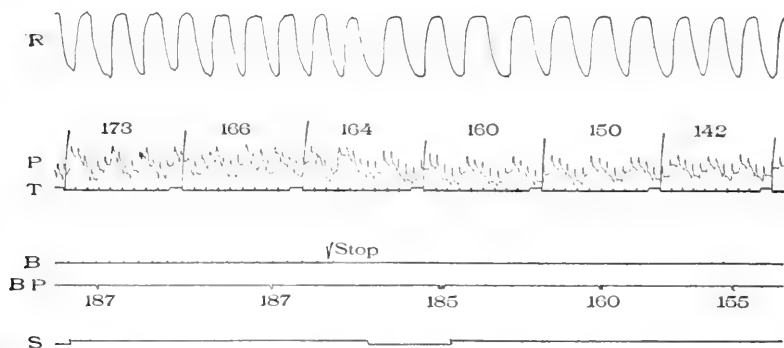


FIGURE 1. — Portion of record taken with slow drums. *R*, respiration; *P*, pulse; *T*, time in seconds; *B*, revolutions of bicycle. *BP*, times at which determinations of blood-pressure were made. The figures written at these points give the pressure in millimetres of mercury. *S*, times at which simultaneous records were taken on quick drum for determination of systole. The part here shown is at the close of a period of work, as indicated by record *B*. Read from left to right.

The duration of systole is determined by recording the pulse along with a fork giving fifty vibrations per second. When this is the only record desired, the outfit just described is used. To determine these small time-intervals accurately the drums carrying the loop of paper must turn rapidly, the entire loop passing the writing points in from one to two minutes. When it is desired to study the duration of systole along with pulse-rate, blood-pressure, etc., during experiments of longer duration, the drums carrying the long loop of paper are turned

slowly, the time being recorded in seconds, and simultaneous records, suitable for determination of systole, are taken on a neighboring drum at any desired intervals. A T-tube is inserted into the rubber tubing connecting the two tambours, and a second piece of tubing leads off from the side branch to a second recording tambour which writes a duplicate pulse-curve for the systole determination.

The blood-pressure is determined by means of the Erlanger sphygmomanometer,<sup>1</sup> with light rubber tubing of one and five-eighths inch diameter for the inner armlet, and a slightly wider strip of sheet brass for the outer one. A wider armlet gives more accurate readings of the absolute pressure, as was first shown by von Recklinghuysen,<sup>2</sup> but one of the width used gives relative pressures with sufficient accuracy and is more convenient. Comparison of readings given with this armlet and a wider one shows that the former gives results from five to fifteen millimetres too high. The pressure determined was the systolic or maximal pressure, as indicated by obliteration of the radial pulse.

To aid the subject in avoiding movements of the neck and arm while at work, since these would interfere with the records to be made, his forehead and shoulders are provided with padded supports, and his left arm, upon which pressure determinations were made, lies in a trough-shaped support; the pedals are provided with toe-clips for the same reason.

#### IV. EXPERIMENTS.

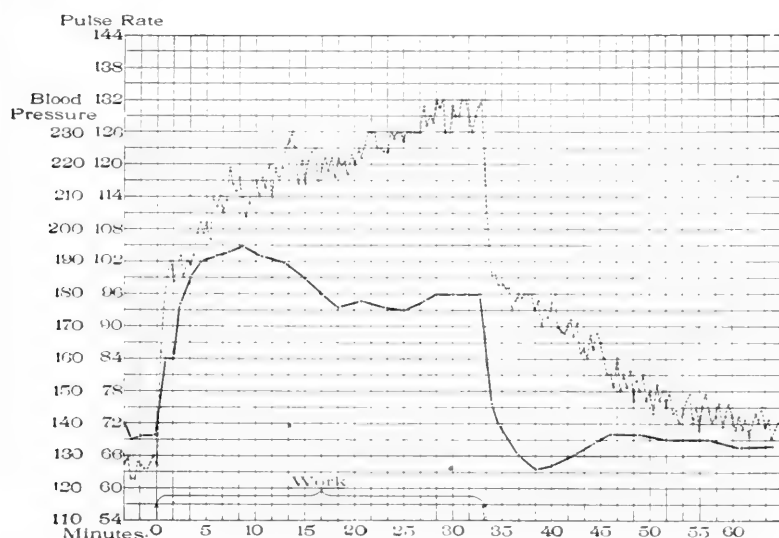
The work done in this series of experiments consisted in driving the stationary bicycle at a constant speed against a constant resistance. The speed and resistance chosen were sufficient to cause a marked but not excessive stimulation of the circulation, and to raise the temperature of the body from one to two degrees: in other words, the work done was just vigorous enough to satisfy the needs of a healthy man who is not in training for athletics. The speed used in most cases was one revolution per second; the resistance, 13.3 kilogram; the amount of work done was approximately 400 kilogram-metres per minute. Note was made of the room-temperature and barometer in each experiment, and the temperature of the body was read at frequent intervals throughout most of the experiments.

<sup>1</sup> ERLANGER: This journal, 1901-2, vi, p. xxii.

<sup>2</sup> VON RECKLINGHUYSEN: Archiv für experimentelle Pathologie, 1901, xlv, p. 78.

The pulse-rate and blood-pressure were taken before, during, and after thirty different working periods on the bicycle. These working periods were of various lengths, the longest being fifty minutes, and the shortest three minutes. In eighteen of the working periods the duration of systole was determined at intervals of one minute or less, in addition to the other items.

Three men served as subjects: H., a chemist, age forty-five; B., a teacher, age forty; and L., a physician, age twenty-five. All these men were in good health at the time, but none was in "training." B. and L. had been accustomed to athletic work in previous years. Successful records were obtained from H. during thirteen working periods; from B. during eleven, and from L. during six periods.



**FIGURE 2.**— Plotted curves showing relative changes in pulse-rate and blood-pressure during thirty-two minutes of work and the thirty minutes of rest following. *P* (broken line), pulse-rate; *B P* (solid line), blood-pressure. Arrows indicate the time of beginning and stopping the work. Experiment made July 8, 1903. Subject, *H*; speed, one revolution of cranks per second; work, 400 kilogram-metres per minute.

The blood-pressure readings were written on the smoked paper, in the proper place, as fast as they were taken (see Fig. 1). After completing the experiment and fixing the curves, the pulse-rate was counted for each interval of ten seconds, and also written on the paper. Both sets of figures were then plotted as in Fig. 2, — time

being the abscissa in each case, and number of beats per minute and blood-pressure in millimetres of mercury being the ordinates. The readings of body-temperature were also written on the smoked paper as they were taken, and afterwards plotted along with the other items, but as we are not fully satisfied with the methods tried, this part of the investigation will be left until a later paper.

#### V. THE CHANGES IN PULSE-RATE.

With regard to the changes in pulse-rate, these experiments corroborate the results of my previous study in every particular. We have in every instance a sudden and rapid primary rise of pulse-rate when the subject begins to work, and unless the work is very light, this is followed by a more gradual secondary rise, which is frequently but not always separated from the former by a plateau, or period of uniform rate. In the case plotted in Fig. 2, the plateau is not present. The secondary rise usually continues until work ceases; then we have a sudden and rapid primary fall of pulse-rate, followed by a slower secondary fall, the two being frequently separated by a plateau.

#### VI. THE CHANGES IN BLOOD-PRESSURE.

The curve representing the changes in blood-pressure resembles the corresponding curve of pulse-rate in having a rapid rise when work begins, and a rapid fall when it stops, but the two curves differ in several important particulars, as can be seen in Fig. 2.

(1) The rise of blood-pressure when work begins follows after the primary rise of pulse-rate, the latter usually being complete in from one to two minutes, while the former continues for four minutes or more.

(2) Instead of rising continuously during the working period, like the pulse-rate, the blood-pressure reaches a maximum after a few minutes, and then declines slowly, with some oscillations, during the remainder of the work. This significant difference in the two curves is of course to be seen only when the work is continued for fifteen minutes or more. The greatest decline observed during a working period was thirty-five millimetres of mercury; in a few instances it was so slight as to be scarcely noticed.

(3) The fall of blood-pressure when work ceases is more rapid than the rise when work begins, but not so rapid as the primary fall of pulse-rate which it accompanies. The rapid fall of pressure, how-

ever, continues to a normal or subnormal figure without interruption, while the primary fall of pulse-rate soon merges into a plateau or a slower secondary fall at some distance above normal. When the pressure becomes subnormal at this time it soon begins to rise again slowly, although the pulse-rate is falling.

## VII. THE DURATION OF SYSTOLE.

In a former paper I called attention to the fact that the quickening of the pulse when work begins is accompanied by a lengthening of systole,—the quickening being entirely due to a shortening of the

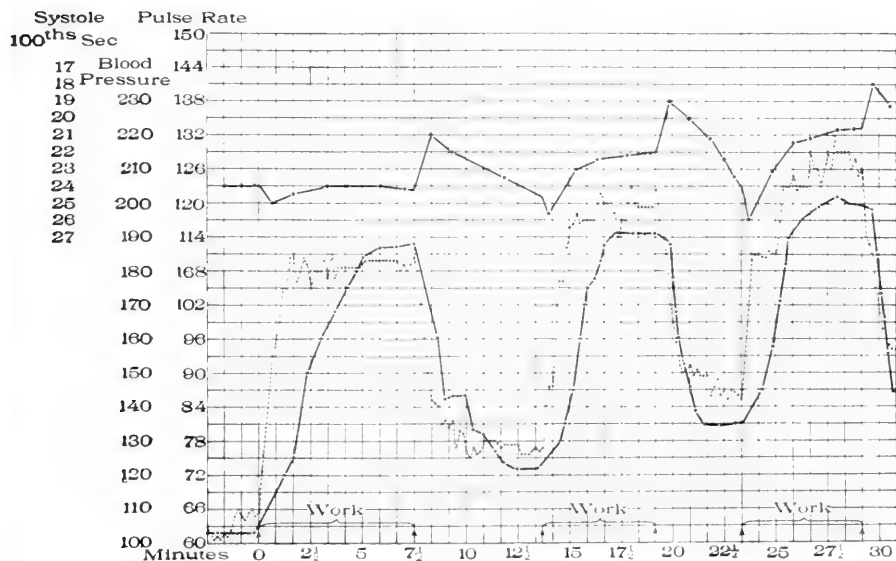


FIGURE 3.—Changes in pulse-rate, blood-pressure and duration of systole during three working periods of five to seven minutes, with four to six minutes' rest between. Uppermost curve, duration of systole; middle curve (broken line), pulse-rate; lowest curve, blood-pressure. Arrows indicate times of beginning and stopping. Time in minutes. Experiment made July 25, 1903. Subject and work same as in Fig. 2.

interval between the beats.<sup>1</sup> In the present research the duration of systole was determined at intervals of one minute or less during eighteen working periods of three to five minutes, and was also determined continuously during eight working periods of about one minute each, and during four experiments in which several short periods of work were alternated with short periods of rest. The re-

<sup>1</sup> BOWEN: *Loc. cit.*, p. 488.

sults clearly verify the former conclusion, although the lengthening of systole when work begins is not equally great in all subjects.

The lowest curve in Fig. 3 indicates the changes in length of systole during three working periods of several minutes each with about four minutes of rest between. In general the systole is seen to shorten during work and lengthen during the intervals of rest,—in full agreement with the results obtained by others. There is, however, a sudden lengthening immediately after work begins, and a sudden and still greater shortening immediately after work stops, in each instance. The curve also shows that the sudden lengthening of systole is at a time when the blood-pressure is beginning to rise, and the sudden shortening is just as the pressure is beginning its rapid fall.

The heavy line in Fig. 4 indicates the changes in length of systole for the successive pulse-beats through a working period of about fifty seconds. The systole lengthens slightly but plainly when work begins, remains longer than normal for about twenty-five beats, then oscillates about the normal until work ceases, when it instantly shortens. The diastole is shorter than the systole during the latter half of the working period.

#### VIII. DISCUSSION OF RESULTS.

**Pulse-rate.**—As I have discussed the causes of the changes in pulse-rate at considerable length in another place,<sup>1</sup> I will not repeat the discussion here, but merely summarize the conclusions as follows:

1. The first and most rapid rise of pulse-rate when work begins is due to an inhibition of the normal restraining action of the inhibitory centre by nervous impulses arising in the motor centres of the cerebral cortex and in the sensory nerve-endings of the contracting muscles.

2. The continuation of the rise is probably due to nervous impulses of like origin acting upon the accelerator centre; to heat and waste products developed in the heart; to heat and waste products developed in the working muscles; and to increased blood-supply to the tissues of the heart through the coronary arteries. The latter may come about through dilation of these vessels, or because of increased pressure in the aorta.

3. The plateau frequently observed is due to a continuation of the

<sup>1</sup> BOWEN: *Loc. cit.*, p. 475.

causes producing the primary rise, the forces involved having reached an adjustment or equilibrium.

4. The secondary rise is due to some of the above causes not becoming effective before, or to changed conditions in the body that render the causes of the primary rise gradually more effective.

5. The rapidity of the pulse during work is much more dependent upon the rapidity of the muscular movements than upon the amount of resistance that is overcome.

**Blood-pressure.** — When one begins to drive the bicycle there are immediately brought into play three causes tending to raise the blood-pressure: (1) increased pulse-rate, as illustrated by the curve of Fig. 2; (2) contraction of the abdominal muscles, which is shown by a change in the respiratory curves, as well as by the fact that there is conscious effort on the part of the subject; and (3) alternate contraction and relaxation of the large muscle groups of the lower limbs, — the latter two causes hastening the return of the venous blood and thus increasing the pulse-volume.

During the progress of the work at least two influences come into play which tend to lower the blood-pressure: (1) dilation of vessels in the working muscles, which has been demonstrated by Chauveau and Kaufmann,<sup>1</sup> Athanasiu and Carvallo,<sup>2</sup> and others; and (2) dilation of vessels in the skin by the agency of the nervous mechanism regulating body-temperature.<sup>3</sup>

Of these two opposing groups of forces, those tending to raise the pressure become effective first and most abruptly; as a consequence, the first effect of the work upon blood-pressure is a rapid rise. But the forces of the second group keep on increasing, — especially the dilation of skin vessels, which probably does not reach its maximum until profuse sweating occurs, from five to ten minutes after starting. Hence the rapidity of the rise of pressure is soon checked, then balanced; finally, when the second group of influences becomes the greater, the pressure begins to fall.

Two other possible causes of diminished blood-pressure during work should be considered: (1) general dilation of vessels through the agency of the depressor nerve, and (2) diminution of the quantity, of venous blood because of sweating. As to the depressor nerve, we have no direct physiological evidence of the existence of this nerve

<sup>1</sup> CHAUCHEAU and KAUFMANN: See Kaufman: *Loc. cit.*

<sup>2</sup> ATHANASIU and CARVALLO: *Archives de physiologie*, 1898, p. 351.

<sup>3</sup> SCHÄFER: *Text-book of physiology*, 1900, i, p. 854

in man; yet its importance in the dog, cat, rabbit, and other mammals, in the absence of any negative evidence in case of man, points to the strong probability that the nerve exists in man, and that it acts during vigorous work to aid in lowering the blood-pressure, and thus protects the heart from overwork. The blood must lose in volume as a result of sweating, but this is gradual, and may be compensated by constriction of splanchnic vessels, as indicated by recent experiments of Hill.<sup>1</sup> Such compensation, however, may not occur during work, when the splanchnic area is under pressure and the systemic vessels strongly dilated.

The variation in the extent of fall in pressure during work in the different experiments was associated with variation in room-temperature, — the fall of pressure being most marked on the warmer days. This variation in the amount of fall is therefore to be attributed to variation in the amount of dilation of skin-vessels. The same variation was observed by Masing, who reports a fall of pressure during work when profuse sweating is present, — not otherwise.<sup>2</sup> This result points clearly to the advantage of "warming up" before attempting to do one's best, as the lowered peripheral resistance lessens the strain on the heart. The advantage of warm weather for athletic competitions is indicated for the same reason.

The more gradual rise of blood-pressure in these experiments, as compared with McCurdy's, can be explained by the different character of the work. It should be borne in mind that this research is a study of the effects of moderate work, such as can be done with safety by the average man; while in McCurdy's, maximal effort was investigated. In the lifting, a greater compression of the splanchnic area results in greater pulse-volume; in the bicycling, the more rapid movements produce a more rapid pulse-rate; both tend to raise the pressure. The difference in the result arises from the difference in the effect of the two kinds of work on the peripheral resistance. In the lifting, the violent and sustained contraction of the muscles nearly obliterates the lumen of the vessels, increasing the peripheral resistance enormously; in the bicycling, the alternate contraction and relaxation of the muscles lessens the peripheral resistance because of the pumping action of the movements on the veins. In lifting, the increased pulse-volume drives the blood-pressure to a great height quickly, because the blood already in the arteries is pre-

<sup>1</sup> HILL: *Journal of physiology*, 1903, xxviii, p. 122.

<sup>2</sup> MASING: *Loc. cit.*



vented from escaping; in bicycling, the faster pulse-rate can force the pressure up but slowly, because of the comparatively open outlet through the capillaries and veins. It seems to me unnecessary to conclude, with Moritz,<sup>1</sup> that there is a direct psychic cause of the rise in pressure in such cases.

On cessation of work, the forces causing the rise of pressure stop as suddenly as they began at the start, while there is delay in the subsidence of those forces which tend to cause the blood-pressure to fall. The peripheral resistance being less than at the start, the pressure falls more rapidly than it rose. Profuse sweating and the associated dilation of skin vessels continues until the body temperature, somewhat elevated by the work, begins to subside, — frequently eight or ten minutes after stopping. Meanwhile the pressure has fallen below the normal level, and now slowly rises as the vessels constrict.

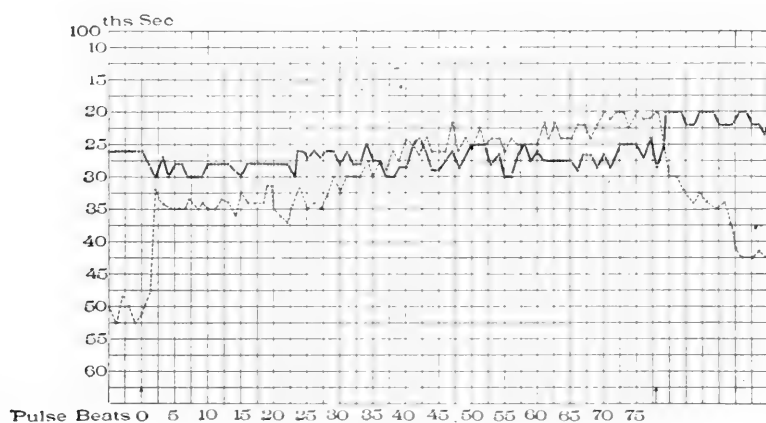


FIGURE 4.—Duration of systole and of diastole during a working period of fifty seconds. Heavy line, duration of systole; light line, duration of diastole. Ordinates indicate duration in 100ths of a second; each unit of abscissa represents one pulse beat; arrows indicate time of beginning and stopping. Experiment made April 17, 1903. Subject, B.; work, about 750 kilogram-metres per minute.

**The duration of systole.** — In accordance with the results obtained by Edgren, Chapman, and others, we are taught that the systole always shortens as the cycle shortens, and always lengthens as the cycle lengthens.<sup>2</sup> When, therefore, my records indicated a lengthened systole at the beginning of work, while the cycle was

<sup>1</sup> MORITZ: *Loc. cit.*

<sup>2</sup> CURTIS: American text-book of physiology, 1897, i, p. 124.

rapidly shortening, I was inclined to consider the result apparent rather than real, and tried to account for the unexpected result in the following ways:

1. As an error of observation in reading the records.
2. As a failure of the tambour to record the pulse-waves correctly, because of its having a vibration period of its own.
3. As a failure to record the pulse correctly because of changes of air-pressure within the tambour system, due to contraction of muscles of the neck.
4. As a possible effect of changes in blood-pressure upon the times of opening and closing of the aortic valves, and consequent change in the apparent length of systole.

After many careful tests of the apparatus, and after observing the same results in nearly one hundred experiments on about thirty different subjects, using tambours of different sizes, levers of various lengths, and membranes of different thickness, it has not been possible to find any reason to suspect the apparatus or the methods of reading the curves.

With regard to the effect of changes in blood-pressure on the apparent length of systole, as indicated by the positions of the primary upstroke and the dicrotic notch, it is clear that increased pressure in the aorta would tend to delay the opening of the valve and hasten its closure, thus rendering our measure of the duration of systole from the pulse-curve too short; similarly, a fall of pressure in the aorta, such as takes place when work stops, would hasten the opening of the valve and delay its closure, and so render the measure too long. In other words, at the time when we observe a lengthened systole, the change in blood-pressure, by its influence on the time of opening and closing of the semilunar valves, tends to make the systole appear shorter than it really is; and when the shortened systole is observed, the blood-pressure is acting to make it appear longer than it really is. The effect of these changes in blood-pressure is thus seen to minimize the real change in duration of systole rather than to account for it.

It should also be taken into account that determination of duration of systole during muscular work was not attempted by former writers, and therefore a change of systole and cycle in opposite directions is not a contradiction of former results, but an observation in a new field.

Taking all into consideration, it seems reasonable to conclude that

the changes in question are real rather than apparent, and the following explanation is offered to account for them:

Soon after work begins we have an increased blood-pressure and an increased pulse-volume. The heart, being obliged to pump an increased volume of blood against an increased resistance before it has been stimulated to stronger action by the accelerator nerves or by increased blood-supply to its tissues, is not able to empty itself as quickly as before. When work ends, the pressure falls, and the heart, having been stimulated to vigorous action, is able to empty itself more quickly than before. A simple pumping engine slows when it meets increased resistance, and quickens when the resistance is lowered. The heart, being primarily a simple pumping engine, behaves in the same way when it suddenly meets a change in resistance, until its complex regulating mechanism can adjust itself to the new conditions and again assume control.

#### IX. PRACTICAL BEARING OF RESULTS.

The practical value of the results of studies in this field depends upon their bearing upon the intensely practical problem of heart-strain. As Roy and Adami have said, "Athletic training is mainly a matter of heart training."<sup>1</sup> We can never lay down proper rules for the guidance of such training until we can determine the changes in heart-strain through a period of vigorous work and the period of recovery from it, since it is through heart-strain that the work most frequently results in injury.

What do we mean by the *heart-strain*? The blood, like all fluids, transmits pressure equally in all directions. During the portion of the cycle when the blood-pressure is at its maximum, the arteries and ventricle are in open communication. It follows that the pressure read upon the sphygmomanometer is not only the pressure in the arteries, but also the pressure exerted by the blood upon each unit of the inner surface of the ventricle. The total amount of pressure thus exerted on the inner surface of the cavity constitutes the *heart-strain*, and is numerically equal to the product of the blood-pressure by the area of the inner surface of the ventricle. The heart-strain varies, therefore, directly with the blood-pressure and with the amount of dilation of the cavity. When from any cause the strain

<sup>1</sup> ROY and ADAMI: Philosophical transactions of the Royal Society, 1892, B, p. 279.

becomes greater than the heart is able to withstand, the result is either, (1) a stretching of the tissue of the heart-walls, so that the dilation does not pass off when the strain lessens (pathological dilatation), or (2) an enlargement of the orifice between the auricle and ventricle, so that the valve cannot close it completely. (Functional incompetence of the valve.) Both these results may occur at once, and in cases of diseased heart-tissue rupture of the wall sometimes takes place. In thus defining the terms used, reference has been made to the left side of the heart, but the principle is equally applicable to the right side.

It must not be inferred that dilation of the heart is essentially an evil. As pointed out by Roy and Adami, the heart tends to dilate whenever its work is increased, as a matter of economy. The economy results, (1) from the fact that any muscle works at an advantage when somewhat elongated, and (2) because the volume of a spherical mass changes faster than its surface, from which it follows that as the heart dilates, the volume of blood pumped out by a certain linear contraction of the heart-muscle increases faster than the strain. It is only when the dilation becomes excessive, in connection with greatly increased blood-pressure, that harm results.

The relation of heart-rate to heart-strain is evidently an indirect one, influencing it only as it influences the height of blood-pressure and the amount of dilation. As the blood-pressure can be determined directly, we need only to consider the bearing of heart-rate upon dilation. Now the faster the heart beats, the less is the tendency to dilation; because (1) by pumping faster it does not need to pump so much blood at each stroke, (2) the nervous regulation causing increased rate causes at the same time increased tone of the heart-muscle, and (3) during muscular work the increased rate is associated with increased force of contraction, favoring more complete emptying. Heitler has observed, in case of the dog, that in general an increased heart-rate is associated with a diminished volume, and a lessened rate with enlargement.<sup>1</sup> We have no evidence, therefore, that a rapid pulse-rate during work is of itself a direct source of danger; it is apparently a protection against overfilling of the cavities and consequent increase of strain.

When work begins, the heart-strain must increase rapidly, because of the great increase in blood-pressure; the fall of pressure during work must tend to relieve it. We cannot tell when the heart-strain

<sup>1</sup> HEITLER: *Centralblatt für innere Medizin*, 1903, p. 625.

is greatest, because the amount of dilation is one of the two factors, and this is at present an unknown quantity. We have an increase in pulse-volume, amount unknown, tending to increase the dilation; an increased rate of beat, tending to prevent it; and fatigue, another unknown factor, tending to increase it. Roy and Adami<sup>1</sup> go so far as to say that fatigue and dilation of the heart may be considered as synonymous terms. When work ceases, we have sudden and great changes in rate and pressure, but the amount of dilation is again an unknown factor, and hence the strain cannot be estimated with any certainty. Before the question of heart-strain can be answered we must be able to state what changes occur in the volume of the heart together with the changes in pressure.

Two principal methods of studying the size of the heart are in use: (1), percussion of the chest wall, and (2), the fluoroscope. The former cannot be used with success during work because of the noise involved. The latter method is far superior in exactness under any circumstances. Schott<sup>2</sup> found a considerable dilation of the heart after vigorous work in wrestling, using both methods. Several scientists have improved the methods of using the fluoroscope in such study. Moritz<sup>3</sup> and De La Camp<sup>4</sup> have failed to verify Schott's results in sound men, but the latter found dilation five minutes after work in cases suffering from disease of the heart. He concludes that maximal muscular efforts cause acute dilation of the heart only when the organ is diseased. Neither of these writers attempted to study the size of the heart during the work. It is to be hoped that extensive studies of the amount of dilation during work will soon be carried on, and preferably in connection with determinations of blood-pressure. Such a study should give results enabling us to determine the conditions which cause the greatest heart-strain, and thus help to put athletic training on a new and firm basis, substituting intelligent methods of training for guess work.

#### X. SUMMARY.

a. By the use of the stationary bicycle the effects of exercises of speed and endurance upon the respiratory and circulatory systems can be studied with accuracy during the progress of the work.

<sup>1</sup> ROY and ADAMI: *Loc. cit.*, p. 282.

<sup>2</sup> SCHOTT: Medical record, 1898, p. 436.

<sup>3</sup> MORITZ: Münchener medicinische Wochenschrift, 1902, p. 1.

<sup>4</sup> DE LA CAMP: Zeitschrift für klinische Medizin, 1903, li, p. 1.

b. During moderate exercises of speed the following changes take place in the circulatory system :

1. A rapid primary rise of pulse-rate when work begins, lasting from one to three minutes; a gradual secondary rise as the work continues, often separated from the primary rise by a plateau; a rapid primary fall when the work stops, followed by a more gradual secondary fall, the two also being separated by a plateau in some instances.

2. A rapid rise of blood-pressure when the work begins, lasting from five to ten minutes; a gradual fall of pressure as the work continues; a rapid fall to normal or subnormal when work stops.

3. A sudden lengthening of systole when work begins; a gradual shortening as the work continues; a sudden and pronounced shortening when work stops, followed by a gradual lengthening to normal.

c. The following explanations are offered to account for these changes :

1. The first changes in pulse-rate when work begins and when it stops are due to nervous impulses acting upon the inhibitory centre. Nervous impulses acting upon the accelerator centre and effects of heat and waste products come in later.

2. The rise of blood-pressure when work begins is produced by increased pulse-rate, contraction of abdominal muscles, and pumping action of the working muscles upon the veins; the latter two causes hastening the return of venous blood and thus increasing the pulse-volume.

3. The cessation of the rise of pressure during work, while the pulse-rate is still rising, is due to diminished peripheral resistance, because of dilation of vessels in the working muscles and in the skin; possibly also aided by the action of the depressor nerve and diminution of blood-volume by sweating.

4. The extent of the fall of pressure during work depends upon the extent to which the skin-vessels are dilated, which in turn depends upon the temperature of the air and amount of clothing worn by the subject.

5. The sudden changes in systole at the beginning and end of work are due to sudden changes in the work of the heart before the regulating mechanism has time to adjust itself to the changed conditions.

d. The practical bearing of these results on the ultimate problem of heart-strain is as follows :

1. A rapid pulse is never an immediate cause of heart-strain, nor a safe indication of how great a strain is present.

2. The changes in blood-pressure during work influence the amount of strain on the heart directly and proportionally, but the time of greatest strain and the general changes in amount of strain cannot be determined without a parallel determination of blood-pressure and change in heart-volume.

3. The maximum heart-strain produced by a certain amount of work is diminished by gradually "warming up" before doing the most vigorous part of it, and is also less when the temperature is warm.

In closing I wish to acknowledge the help given me by Dr. Warren P. Lombard, under whose direction the work was carried on; I am also indebted to Mr. G. O. Higley, Instructor in Chemistry in the University of Michigan, and Dr. William Litterer, Instructor in Physiology in Vanderbilt Medical School, for valuable assistance in making the experiments.





## ON THE ACTION OF LOBELINE.

By CHARLES W. EDMUNDS.

[*From the Pharmacological Laboratory of the University of Michigan.*]

THE question as to who introduced *Lobelia inflata* or Indian tobacco into medicine is one that is hard to answer. The drug had, no doubt, been used for a long time by the laity before it began to be employed by regular practitioners, and it is possible that it was used by the Indians before the time of the whites, although opinions differ on the subject.

The first account of the emetic property of the drug was published in 1785, by the Rev. Manasseh Cutler,<sup>1</sup> who began to employ it in asthma in 1809. Schoepf<sup>2</sup> in 1787, writing on the subject, confused *Lobelia inflata* with *Lobelia syphilitica*, and ascribed the properties of each drug to the other.

The name which above all others is most closely connected with *Lobelia inflata* is that of Dr. Samuel Thomson, the founder of the Thomsonian system of medicine; under this system, lobelia was the most important member of the class of "Emetics," and was employed so extensively by Thomson and his followers that they were very commonly called "Lobelia doctors."

The origin of the common name "Indian tobacco" is much disputed. According to J. U. and C. G. Lloyd<sup>3</sup> (to whom I am indebted also for a large part of the early history of the plant), it was first called "Wild tobacco," and from this it was very easy to have the name changed to "Indian tobacco," as it would naturally be thought that a tobacco growing wild would be used by the Indians. However, the Lloyds say all the evidence they have been able to collect is against the theory of its use by the aborigines. Carver, Lewis and Clark, and Zina Pitcher, and many others who have

<sup>1</sup> CUTLER: American Academy of Sciences, 1785, i, p. 484.

<sup>2</sup> SCHOEPP: *Materia Medica Americana*, 1787, p. 128.

<sup>3</sup> J. U. and C. G. LLOYD: *Drugs and medicines of North America*, 1886, ii, p. 65.

written on the subject of Indian remedies, do not mention "*Lobelia inflata*." The one exception is Mattson<sup>1</sup> (1841), who states that there is abundant traditionary evidence that it was used by the Penobscot Indians long before the time of Dr. Thomson. With this one exception, the evidence all seems to be against the use of this herb by the early inhabitants of this country.<sup>2</sup>

As lobelia was brought before the public so prominently in various trials of the Thomsonians for murder and manslaughter in which it figured, it was natural that it should be examined as to its chemistry, and later as to its pharmacological action.

One of the first important pieces of work upon the pharmacological side of the question was by Ott<sup>3</sup> in 1875. He was followed by Ronnberg<sup>4</sup> in 1880, Bartholow<sup>5</sup> in 1886, and by Dreser<sup>6</sup> in 1889, and still later by Bliedtner<sup>7</sup> and Tietze.<sup>8</sup>

There still exists some question as to the position of lobeline in the pharmacological system, and I have attempted to determine this more satisfactorily in the following series of experiments. It very soon became apparent that its nearest affinity was to the group of nicotine (Langley and Dickinson<sup>9</sup>), and accordingly the effects of these two alkaloids were in many cases contrasted in parallel experiments.

The alkaloid was extracted from the powdered seed of *Lobelia inflata* (kindly supplied me by Parke, Davis, and Co.), the method employed following that described by Dreser.

The powdered drug was percolated with sixty per cent alcohol acidulated with acetic acid, and the resulting tincture concentrated on a water bath, precipitated by lead acetate and filtered. The excess of lead was removed

<sup>1</sup> MATTSON: American vegetable practice.

<sup>2</sup> The origin of the popular name is of some anthropological interest, since the action of *Lobelia* resembles that of tobacco so closely. A similar interest attaches to the habit of chewing pituri among the Australian natives, as LANGLEY and DICKINSON (Journal of physiology, 1890, xi, p. 265) have shown that the action of piturine is practically identical with that of nicotine.

<sup>3</sup> OTT: Philadelphia medical times, 1875, vi, p. 121.

<sup>4</sup> RONNBERG: Inaugural dissertation, Rostock, 1880.

<sup>5</sup> BARTHOLOW: Drugs and medicines of North America, 1886, ii, p. 89.

<sup>6</sup> DRESER: Archiv für experimentelle Pathologie und Pharmakologie, 1889, xxvi, p. 237.

<sup>7</sup> BLIEDTNER: Inaugural dissertation, Kiel, 1891.

<sup>8</sup> TIETZE: Inaugural dissertation, Griefswald, 1903.

<sup>9</sup> LANGLEY and DICKINSON: Journal of physiology, 1890, xi, p. 265.

nervous system and peripheral nerve terminations. Muscular twitchings may be present.

From this brief description it will be seen that the main difference between the two drugs is that with lobeline the rigidity is not found, indicating the absence of the stimulant action on the hind brain.

#### EFFECT ON WARM-BLOODED ANIMALS.

To a small rabbit Ott gave one drop of "*Lobelina*," intravenously, resulting in struggling and death in one minute. In another animal subcutaneous doses of small amounts had little effect, while an intravenous injection caused convulsive movements and spasms of head and jaw muscles. Dreser gave five milligrams to a cat, the injection being followed by struggling, vomiting, and dyspnoea. Later, one hundred milligrams caused death in forty minutes from a central paralysis of the respiration.

Ronnberg got very much the same effects in dogs.

**Personal observations.**—In the experiments upon this phase of lobeline action symptoms resembling those described were obtained as well as some not mentioned above. Small doses (one-half to five milligrams) of lobeline, given subcutaneously, caused violent vomiting in cats in from three to three and one-half minutes, this vomiting being repeated several times during the next ten minutes. About three-quarters of an hour later it came on again, but this time the vomitus was white and foamy, being composed entirely of swallowed saliva and mucus. Twitching of the ears appeared to be a constant symptom, coming on three or four minutes after the drug was given. At first the ears usually moved together, but later they twitched in opposite directions. This twitching does not last for more than about a minute, but it may come on again later, though it is usually less marked and for a shorter time. The animal often urinates, and movements of the bowels are not infrequent. Other symptoms found, such as distress, unrest, and depression, are probably secondary to the emesis.

To see if the twitching of the ears was caused by the drug, or was due to the vomiting alone, other experiments were carried out on cats in which emesis was caused by apomorphine; but in no animal did any twitching of the ears take place, showing that with lobeline it was not secondary to the vomiting, but was due to some direct action.

reflex excitability, followed by loss of will power, with lessened excitability, passing on to final total paralysis. Dreser localized the spinal cord as being the seat of the increased reflex excitability, and also demonstrated a curara action on the motor terminations.

**Personal observations.**—The results obtained upon this animal add little to the work given above, but in the main confirm it. After the injection in a large frog of three milligrams of lobeline, the animal made attempts at jumping, but in a few minutes became quiet. The respiration was markedly slowed and irregular, and in some cases all visible respiratory movements ceased. The animal remained quiet unless it was disturbed by pinching, when it would hop away, the movements being clumsy and not well co-ordinated and there being some difficulty in regaining the normal position. When placed on its back, it was usually able with some effort to right itself. After some hours, during recovery, an increase in reflex excitability was at times noticeable.

With doses of five to ten milligrams, the animal showed more difficulty in its bodily movements, with complete loss of control over its forelegs, causing it to rest on its abdomen. It would not hop away when disturbed, and reflex movements finally disappeared, total paralysis coming on, due to a curara-like action on the motor terminations. This paralysis, after a day or two, began to pass off, and the animal exhibited marked reflex excitability resulting, in some cases, in strychnine-like convulsions. The convulsive contractions were usually not as prolonged as would be caused by strychnine, but resembled more those in which curara and strychnine are given together. In several cases the frogs died two or three days after the lobeline had been administered, and none of these later effects were obtained.

In his experiments on frogs, Ott reported "muscular twitching and convulsive movements of extremities," but although I have looked carefully for such muscular phenomena, I have never seen them, but think their absence might possibly be explained by the variety of frogs used (*Rana virescens*), or perhaps by the season of the year influencing them. This is made more probable by the fact that in the experiments made with nicotine as a comparison, I was not able to get any twitching whatever.

Langley and Dickinson state that nicotine first causes a state of excitement in the frog. This is followed by a characteristic rigid posture which gradually passes off through paralysis of the central

nervous system and peripheral nerve terminations. Muscular twitchings may be present.

From this brief description it will be seen that the main difference between the two drugs is that with lobeline the rigidity is not found, indicating the absence of the stimulant action on the hind brain.

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With larger doses (fifteen to thirty milligrams) the symptoms differ more in degree than in kind, the dyspnœa and depression being much more pronounced. The animal moans and cries, staggers about, falls forward with head on the floor, the trouble with walking being especially noticeable in the hind limbs. With these large doses, the vomiting is not as marked as with the smaller doses. In some cases it occurred as early as with the small doses, but was not repeated more than once or twice. In other animals it did not come on for ten or fifteen minutes, and then was not repeated. Micturition and purgation are more common symptoms—an involuntary diarrhœa not being unusual.

A dose of seventy-five milligrams given subcutaneously to a cat of 1250 grams caused violent panting in twenty seconds; the animal cried, ran about the cage, its ears twitched for about a minute and then lay back close to the head, and the hair on its tail was raised. In two and one-half minutes the animal was panting with head lowered, twitching and trembling all over. In six minutes a violent convulsion came on, tonic in character, lasting about twenty seconds. Its body was rigid and its legs were extended stiffly at right angles to the body. The animal made violent leaping movements two or three inches in the air. After the convulsion, the animal lay on the bottom of the cage, trembling violently, but later the attacks of trembling came only every five or ten seconds. Four minutes after the first convulsion, another came on of much the same character, except that the cat lay on the bottom of the cage. This was followed by feeble kicking movements, and the animal died fifteen minutes after the injection had been made. It will be noticed that no vomiting whatever occurred in this animal. With the increasing size of the dose, this symptom appears to become less prominent.

The emesis is no doubt due to stimulation of the vomiting centre, as has been claimed by the earlier workers, because, given by the stomach, larger doses are required and a longer time to act.

In a mouse marked tremors were seen after small doses of lobeline, with jerking of the anterior part of the body, followed later by generalized twitchings and tremors that became more severe until the respiration stopped.

The experiment in which seventy-five milligrams were given to a cat shows that, contrary to some of the earlier workers, lobeline has a marked effect on the central nervous system when given in suffi-

ciently large doses. It may be added here that in those cases in which fifteen to twenty-five milligrams were given, with the usual symptoms of dyspnœa, etc., if a second dose of the same size was given, half an hour to an hour later, the symptoms were not nearly as marked as at the primary dose, the vomiting and dyspnœa, perhaps, being entirely absent. An explanation of this might be that the depression of the nervous system following the stimulation of the first dose was so great that the nerve cells could not react to the new injection.

In rabbits, subcutaneous injections of from five to seven milligrams had little effect beyond causing a marked slowing of the respiration. At times the animal appeared rather more restless than usual, and seemed to lose partial control of its limbs, as when resting it would often slide its fore limbs out in front of it, and the hind limbs would be stretched out backwards, instead of drawn up closely under it, as is normal.

In dogs, the effect is much the same as in cats. One milligram given subcutaneously to a small dog caused no symptoms beyond a markedly accelerated respiration. Three milligrams, in addition to the respiratory changes, caused vomiting in fifteen minutes. Ten milligrams given subcutaneously to a large dog increased the respiration and caused emesis in five minutes.

According to the observations of Bernard, V. Praag, Krocher, and Truhart (cited by Langley and Dickinson), nicotine causes, in mammals, a preliminary excitement, clonic spasms and twitching of the muscles in various parts of the body, and at times convulsions and opisthotonus. In the cat, there occurs a certain rigidity of the fore limbs (V. Anrep.) and twitching of the ears (Truhart).

Langley and Dickinson found the motor nerve ends were paralyzed in a cat by ten to fifteen milligrams, but that a larger amount was required in the rabbit and very much more in the dog.

One difference between the two alkaloids would seem to be that lobeline does not cause a preliminary excitement or clonic spasms with muscular twitching (with the exception of the action on the ears), unless it is given in very large and rapidly fatal doses, when these symptoms may occur. The emetic power of lobeline would appear greater than that of nicotine. The paralysis of the motor endings has not been demonstrated in mammals from lobeline action.

## RESPIRATION.

As to the effect of lobeline upon the respiration, Ott said, the drug was a respiratory poison, as the heart beat after the respiratory movement had stopped. He also said, the respiration is first accelerated and then depressed, and these changes not taking place after section of the vagi, he attributed them to irritation of the endings of these nerves in the lungs. Dreser carried out the most extensive work upon this part of the lobeline action, and summarized his results as follows: lobeline produces powerful excitement of respiratory activities; the respirations are increased in frequency, this lasting longer with vagi intact than when they are cut; the volume of each respiration is increased as well as the force with which the breathing muscles are innervated from the centre, resulting in increased amount of work; the contractile effect of the vagi upon the bronchial muscles becomes non-effective after relatively small doses. Dixon and Brodie<sup>1</sup> have recently stated that a preparation of lobelia causes some constriction of the bronchial tubes, unless where these are already in a state of contraction, when the lobelia injection is followed by a transient dilatation.

**Personal observations.** — In a cat, not under an anæsthetic, one milligram subcutaneously caused the respiration to become not only accelerated, but also irregular, but this irregularity is probably caused by the marked nausea. With larger doses (15 milligrams for cat of 2400 grams) the respiratory rate was very greatly accelerated; in three or four minutes it was thirty-five in fifteen seconds, the animal standing with its mouth wide open, violently panting. Five minutes later the rate had dropped to fourteen in fifteen seconds, and still later to twelve, and at the end of half an hour to nine. During this period the animal had short attacks of dyspnœa, violent panting, with the mouth open.

A subsequent injection of ten milligrams given fifty-five minutes after the primary fifteen milligrams caused practically no acceleration whatever. There was, however, a progressive slowing from eleven before the injection to eight four minutes after, remaining at this rate for about fifteen minutes, when it increased slightly. With rabbits that were not anæsthetized the investigations of the effects on the respiration were very unsatisfactory, as external influences seemed

<sup>1</sup> DIXON and BRODIE: *Journal of physiology*, 1903, xxix, p. 169.



to be such a large factor in the animal's breathing. However, a dose of seven milligrams given subcutaneously caused very marked slowing, decreasing the rate to almost one-half the normal.

In a mouse, one-half milligram given subcutaneously caused first acceleration, dyspnœa, and irregularity followed by slowing, which began about three minutes after injection, and lasted for about three-quarters of an hour. In subsequent injections of doses from one to two milligrams, the acceleration became less marked than in the primary injection, but the slowing became greater until the respiration ceased entirely after a total of five and one-half milligrams had been given in an hour and a half. In anæsthetized animals there was found the acceleration followed by slowing which has been described.

In a dog under morphine and chloretone, one or two milligrams caused marked respiratory movements like those seen in cats, but a second dose of the same size caused none of these effects. After the dyspnœic breathing following the primary injection of five milligrams, all respiratory movements ceased for possibly twenty seconds, when artificial respiration was begun, but in a very few seconds the natural process was reinstated. Apparently the first dose stimulates the respiratory movement, but its secondary effects antagonize those of subsequent doses. This antagonism is interesting because, although the centre is incapable of responding to further stimulation, it does not seem to be incapacitated for its ordinary function, for the rate is not markedly slowed.

Nicotine causes a quickening and deepening of the respiration (V. Praag and Rosenthal). In anæsthetized animals the drug first causes slowing and deepening of the respiration, followed by an accelerated rate (Langley and Dickinson). Section of the vagi lessens the quickening effects of the drug, but deepens the respiration.

There is, therefore, very little difference between the two drugs in their effects upon the respiration, the changes caused by them probably differing more in degree than in kind.

#### BLOOD-PRESSURE AND HEART.

The effect of lobelia upon the circulation has been studied most carefully by Ott, who found that in dogs and rabbits the drug caused a temporary fall of pulse-rate and blood-pressure. This was succeeded by a rise in pressure and increase in pulse-rate beyond the

normal, and occasionally before death the pulse became exceedingly rapid. Furthermore he says, section of the vagi or paralysis of them by nicotine or atropine did not prevent the same sequence of events from taking place, excepting that where nicotine was used the rise in pressure was very slight. He located the point of action of the drug in the excitomotor ganglia in the heart which have their excitability diminished. He added further that lobeline paralyzed the vagi and also the vasomotor centre, and the rise in pressure was due to spinal or peripheral vasomotor action.

Ronnberg obtained a rise in pressure up to two minutes after the injection of one milligram in the jugular vein. This was followed by a decrease in pressure for five minutes, and after this decrease, for five or ten minutes, a regular rise and fall in pressure with a small frequent pulse. The latter changes coincided with periodic vomiting. From the fact that lobeline removed the muscarine standstill of the heart, and also that in lobeline-poisoned hearts muscarine either does not act at all, or acts only in larger doses, he concluded that lobeline acted on the peripheral endings of the vagi.

**Personal observations.** — Most of my work upon the effect of lobeline upon the circulatory system in mammals was carried out upon cats anesthetized with morphine and chloretone, — a cannula connected with the recording manometer being inserted in the carotid, and the injections made into the jugular vein.

An analysis of the tracings obtained confirms, in the main, Ott's work, but with some variations, which will be noted.

The changes taking place in the circulation may perhaps be most simply described in three stages. 1st. A stage of bradycardia and low pressure. 2d. Acceleration of the pulse and rise in blood-pressure. 3d. Gradual decline in both pulse-rate and blood-pressure.

*Stage 1.* — About five seconds after an injection of one milligram of the drug, there was marked slowing of pulse, with finally a complete stoppage of the heart, lasting from one to two seconds. This cessation was followed by two or three irregular contractions, and then, for the next ten seconds, regular strong beats at less than half the normal rate, followed by gradually accelerated rhythm as will be described under Stage 2.

The blood-pressure changes during this period of bradycardia were quite marked; it fell gradually, becoming irregular during the period of stoppage, and arrhythmia of the heart; but during the slow regular

beats the pressure is very much lower than normal, but regular with the pulse-wave very large. This stage of slow heart and low pressure passes then into Stage 2.

*Stage 2.* — The heart is gradually but rapidly accelerated, and the blood-pressure greatly raised, reaching the highest point in from thirty to forty-five seconds after the drug is given. The pulse, when it is most rapid, is about half as fast again as normal, and the pressure very much higher than the normal.

*Stage 3.* — This stage lasts for from four to five minutes, during which time the pressure and pulse-rate slowly and gradually fall, until both have reached the normal, or more often may be slightly below the normal.

The effect of subsequent doses differed. A smaller dose (five-tenths milligram) than the original caused no change in either pulse-rate or pressure. A dose of the same size as the original (one milligram) gave a curve much the same as that described under Stages 2 and 3 above. In other words, Stage 1 was absent. The rise in pressure was not as marked as in the primary injection, and the subsequent fall was more marked, that is, it reached a lower point each time, as described below.

Injections given later varied in their effect, the rise in pressure becoming less marked after each injection, until, after two or three doses, no rise would result. The acceleration of the pulse also became less marked. The decline in pressure at the end of Stage 3 also ended after the first few injections, with the height of pressure slightly lower than it had been after the preceding injection, as mentioned above. This fall was only down to a certain level which would be maintained after later injections.

As an example of the variation in pressure occurring from repeated doses, the measurements from one experiment may be given, the figures being the height of the curve from the base line.

Before injection.	Dose.	Highest point reached.	Height at end of decline.
millimetres.	milligrams.	millimetres.	millimetres.
52	1	86	46
46	1	53	42
42	2	49	39
39	3	42	39

If the primary dose was larger (five milligrams), the effect was much the same as we have described, with at times some irregularity of the heart during the decline in pressure. The greatest difference, however, was seen with subsequent injections, which usually gave no change in rate or pressure.

Several experiments were carried out in order to locate, if possible, the points of action of the drug. A cat was prepared as before, and in addition the vagi were severed in the cervical region. Injections of lobeline then gave a slight slowing and a small fall in pressure, but neither effect was as marked as in experiments with intact vagi. Stages 2 and 3 followed as before. In another cat both vagi were paralyzed with atropine before the drug was injected. In this case there was no slowing of the heart or fall in blood-pressure, while the other phenomena were not affected. These results clearly show that the preliminary fall in both pulse and pressure is due partly to central and partly to peripheral action on the vagi.

It is well known from Langley's, Schmiedeberg's, and Truhart's researches that nicotine first stimulates and then paralyzes the sympathetic ganglia on the vagus nerves, in addition to acting on the medullary centres, and it seemed not unlikely that lobeline might act in the same way, as the pressure-curves obtained from it were almost identical with those given by nicotine. To make the explanation still more probable was the fact that lobeline paralyzes the superior cervical ganglia, as will be shown in the discussion of its action on the pupil.

Nicotine was accordingly administered to a cat, until practically no change in blood-pressure resulted from its injection, and lobeline was then given, resulting in a very slight fall and subsequent slight rise, resembling exactly the phenomena shown under nicotine action. In other experiments, alternate injections of the two drugs gave curves which it would be impossible to distinguish from one another.

Against this explanation, there is Ronnberg's work, showing that the muscarine standstill of the frog's heart is removed by lobeline, and this effect was confirmed in my work. I also found that five milligrams of pilocarpine would not stop a cat's heart after seven and one-half milligrams of lobeline had been given. There resulted only a slowing in the heart from one hundred and eighty beats in a minute to one hundred and fifty.

It would appear necessary, therefore, to ascribe to lobeline some action on the extreme terminations of the inhibitory nerve, as well as

on the ganglia on their course, but it seemed unlikely that it possessed both. The difficulty was finally cleared up by the examinations of the effects of the drug upon the hearts of cold-blooded animals. These were examined first in the turtle, upon which I carried out a number of experiments designed especially to elucidate the relation between lobeline on the one hand and pilocarpine and atropine on the other.

The brain of a turtle having been destroyed, the plastron was removed, the pericardium opened, and the two sides of the exposed ventricle were connected to a myocardiograph.<sup>1</sup> The lobeline solution was injected in some cases into the vessels, while in others it was dropped on the heart. In general it might be said that after lobeline had been given pilocarpine had practically no action in slowing the heart. If, on the other hand, pilocarpine had been given first, bringing the heart to a standstill, and lobeline was then applied directly to the organ, it began to beat again after some time (ten or fifteen minutes), and the rate gradually increased, but in no case did it return to the normal. If, later, atropine was given, the rate was still more increased, but even then it did not return to the original rate. An objection might be made to these experiments that the heart was connected to the instrument by threads through its wall, subjecting it to injury and constant irritation, which might account for the changes in rate found. To avoid this, in another turtle no apparatus was used, the rate of the heart being counted with the following results.

*Experiment. —*

Time.	Rate per minute.
2.50	24.
2.55	24.
2.58	Two mgms. pilocarpine injected into aorta toward the heart. The heart stopped at once.
3.03	Two and one-half mgms. lobeline into aorta peripherally, two cm. from heart.
3.15	Occasional feeble beats.
3.20	Rate 5 per minute. — Contractions weak.
3.32	Rate 5 per minute. — Contractions weak.
3.35	Few drops lobeline solution direct to heart.
3.39	Rate 5. — Contractions strong.
3.45	Rate 6.

<sup>1</sup> CUSHNY and MATTHEWS: *Journal of physiology*, 1897. xxi, p. 213.

*Experiment (continued): —*

3.55	Rate 7.
3.57	Rate 8.
4.10	Rate 8.
4.15	Two mgms. pilocarpine into artery.
4.20	Rate 9.
4.25	Rate 8.
4.28	Atropine to heart.
4.31	Rate 7.
4.34	Rate 26.
4.40	Rate 26.

Lobeline, when given either by injection into the frog's lymph sac, or by direct application to the heart, causes a progressive slowing and weakening of the organ. These effects, as in the case of the turtle, are not removed by atropine, and therefore are of muscular origin.

If atropine is applied to the hearts of either frogs or turtles, and subsequently lobeline is used, there soon follows a slowing of the heart, with weakening of the contractions, these effects being due to a direct action on the muscle itself. As to the relation of lobeline to muscarine, I found, as stated above, that Ronnberg's statements as to the removal of the muscarine standstill were correct. However, in none of his experiments did he use atropine later, to see if that would still further quicken the heart after lobeline. He could not tell, therefore, if the nerve-terminations, which had been stimulated by the first drug, had been paralyzed by lobeline, which would be necessary if it had neutralized the muscarine action. In only one of his experiments (Table VII, No. VI) did the heart return to the normal rate, and then not until two hours after lobeline had been applied.

In all my experiments, while lobeline started the heart after it had been brought to a standstill by muscarine, it did not return it to the original rate. If atropine was then given, the rate was increased at once, and in many cases it reached the normal. In other cases, where larger amounts of lobeline had been used, atropine quickened the heart, but did not cause a return to the normal rate. Conversely, if lobeline was applied to the heart, and later muscarine was given, some slowing from the latter drug usually resulted, but in no case did it stop the heart entirely. The slowing was, of course, removed by atropine.

These changes in the rate under lobeline, pilocarpine, and atropine are well shown in the turtle experiment given in detail above. The heart, which had been contracting at the rate of twenty-four per

minute, was brought to a standstill by pilocarpine; lobeline brought the rate up to eight per minute, which seemed to be the limit of its action, but atropine caused a return to the normal.

These facts would lead us to suspect at least that lobeline does not act on the vagus-terminations as does atropine, because, if it paralyzed them, atropine could do no more, and therefore should not increase the rate of the heart over what it was under lobeline. This action of lobeline, then, inhibiting or preventing the muscarine and pilocarpine standstill, I would ascribe to an action upon the heart-muscle itself changing it in some obscure way, so that these drugs could not have their customary effects upon it. It may not be inappropriately compared to that of physostigmine, which also acts upon the heart-muscle of cold-blooded animals in such a way that it partially removes the muscarine standstill (Harnack).

The supposition that lobeline acts upon the sympathetic ganglia on the course of the vagi, and not upon the extreme terminations was proved by the fact that while in the frog, after lobeline had been given, stimulation of the vagus had no effect, yet stimulation of the sinus of the heart stopped the heart, proving that the nerve-terminations were intact.

It may be worth while to mention that in no experiment carried out on rabbits or cats has been seen the great rapidity of the heart described by Ott as occasionally found just before death.

The acceleration of the heart which occurs in the second stage described, is not affected by a previous injection of atropine, so that it must be due either to stimulation of the accelerator-nerve, or to direct action on the muscle. The fact that it only occurs after the first few injections (sometimes after only the first), would make the former explanation more likely; that is, that the accelerator mechanism is first stimulated and then paralyzed. That the inferior cervical ganglion, from which the accelerator fibres arise in the dog, is thus acted on by the drug will be shown later in the paper.

Lobeline then stimulates the cardio-inhibitory centre in the medulla, and first stimulates and then paralyzes the ganglia on the course of the vagi. It also stimulates and then paralyzes nerve cells on the course of the cardiac accelerator fibres, and this stimulation is the cause of the increased rate of heart seen thirty seconds to a minute after the injection of the drug.

Its action on the regulating apparatus of the heart would seem to be identical with that of nicotine. In addition, lobeline acts upon the

heart-muscle of cold-blooded animals in such a way as partially to remove or overcome the action of such drugs as muscarine upon that organ.

#### EFFECT ON THE ACCELERATOR NERVE OF THE HEART.

A large dog was anæsthetized with morphine and chloretone, and a tracheal cannula having been inserted, artificial respiration was started, the chest opened, and the heart exposed. The right auricle and ventricle were then connected with a myocardiograph. The inferior cervical and stellate ganglia, with the annulus, were dissected out, together with the cardiac branches from the inferior cervical ganglion and from the annulus. Electrical stimulation of the various nervous structures mentioned, brought out typical accelerator effects. After five milligrams of lobeline had been injected intravenously, stimulation of the annulus had no effect, while stimulation of the cardiac branches from the inferior cervical ganglion accelerated the heart in the usual way. Lobeline, therefore, paralyzes the accelerator nerve by acting on the inferior cervical ganglion. Before the ganglion is paralyzed, it is first stimulated, as is shown by the exceedingly rapid heart following the paralysis of the vagus ganglia; but that the rapid heart is not due to the vagus paralysis, is shown by the fact that it also occurs if the vagi have been paralyzed by atropine previous to the injection of lobeline, leaving the accelerator nerve and the heart-muscle as the possible seats of action. It does not seem likely that it is due to direct muscular action, because the acceleration only occurs after the first one or two injections of the drug, after which, as has been pointed out, the inferior cervical ganglia are paralyzed.

Langley and Dickinson believe the acceleration under nicotine is due to a similar action on the ganglia, but this is denied by Wertheimer,<sup>1</sup> who reported acceleration even after extirpation of both stellate ganglia, and therefore believes it is due to the muscular action. This is denied by Dixon,<sup>2</sup> who found that nicotine caused no acceleration of the heart after the ganglia had been paralyzed by apocodeine.

In its action on the accelerator nerve of the heart, lobeline has the same effect as nicotine, as the latter drug also causes a primary stimulation of the sympathetic ganglion on the course of these fibres, followed by paralysis of the same structure.

Lobeline, however, differs from apocodeine, which also paralyzes this

<sup>1</sup> WERTHEIMER and COLAS: *Archives de physiologie*, 1891, xxiii, p. 341.

<sup>2</sup> DIXON: *Journal of physiology*, 1903, xxx, p. 105.



ganglion, but without any preliminary stimulation (Dixon<sup>1</sup>). Moreover, apocodeine, in addition to acting on the ganglion, also paralyzes the extreme terminations of the accelerator fibres in the heart-muscle (Dixon). This effect, which is seen after apocodeine only when the drug is given in large doses (250 to 300 milligrams), has not been shown to take place with either nicotine or lobeline.

#### BLOOD-PRESSURE.

To localize the point of action of the drug which resulted in the rise of blood-pressure, the following experiments were carried out. A cat was prepared as heretofore, and in addition its spinal cord was cut across at the level of the fourth cervical vertebra. Injections of the drug were followed by the rise of pressure as before. In a second animal, the spinal cord was entirely removed from the fourth cervical to the fourth lumbar vertebra, yet the injections of lobeline resulted in a rise, the same as before, despite the severe state of shock following the operation.

These experiments proved beyond all doubt that the seat of action is not central or spinal, but is on the peripheral vasomotor system. It was impossible to ascertain just what peripheral structure on the course of the vasoconstrictor fibres was acted on, as these fibres differ from the dilators in having no definite ganglia in which they all end on sympathetic cells, as the white rami of the constrictors may pass through several ganglia before reaching their termination. Therefore, although I think there can be no doubt that the drug acts on the ganglia, first stimulating and then paralyzing them, as has been shown it does elsewhere, this view could not be proved, as at no point could I cut the trunk of the nerve and be sure I was stimulating all spinal or all sympathetic fibres.

Nicotine first stimulates and then paralyzes the peripheral nerve cells on the course of the vasoconstrictor and vasodilator nerve fibres, the total result being a primary great increase of blood-pressure, caused by constriction of the vessels in the splanchnic area, followed after three minutes by a fall below normal. The similarity of the blood-pressure curves obtained under lobeline, and the fact that the effects of each drug are weakened by the previous injections of the other, indicate a common seat of action.

<sup>1</sup> DIXON: *Loc. cit.*, p. 98.

## VASOMOTOR EFFECTS.

Ronnberg says the vessels of a rabbit's ear are dilated by lobeline through paralysis of the sympathetic nerve.

**Personal observations.** — For this work white rabbits, anæsthetized with paraldehyde, were used, injections of the lobeline being made into the jugular vein, with the following results. Immediately after the injection of small doses, the ear showed a marked pallor, which lasted for from two to three minutes, when it began to get pinker, and gradually became markedly flushed, this flush lasting for ten or twelve minutes, at the end of which time the ear had returned to its normal hue. Secondary injections, made some time after the first, gave the same results as the primary, except that the effects, especially the pallor, were not quite as marked as after the first.

Extirpation of the superior cervical ganglion modified the phenomena, in that there was no primary pallor in the ear on the side on which the ganglion had been removed. Instead, there was a flushing, appearing almost immediately after the drug was injected, this flushing lasting for five or seven minutes, when it gradually passed off. This flushing is probably a secondary result of the general constriction of the vessels, the blood flowing in greater abundance than usual into the unconstricted vessels of the operated ear. During the time when both ears were flushed, the ear on the side on which the ganglion was intact was never as deeply flushed as on the side on which the ganglion had been extirpated. The preliminary pallor in the intact ear is then due to the stimulation of the superior cervical ganglion, which distributes vasoconstrictor fibres to the vessels near the base of the rabbit's ear (Meltzer<sup>1</sup>).

The changes in the circulation in the rabbit's ear caused by nicotine are almost identical with those caused by lobeline.

## CIRCULATORY CHANGES IN THE MESENTERIC AND RENAL VESSELS.

To study the changes in the circulation in the abdominal vessels, the changes in the blood-content of the intestine and in the volume of the kidney were recorded, the general blood-pressure being taken from the carotid at the same time in the usual way; cats anæsthetized with morphine and chloretone were employed in the experiments.

<sup>1</sup> MELTZER: This journal, 1903, ix, p. 57.

To measure the changes in the intestinal vessels, a plethysmograph was used, made in plaster of Paris after a pattern described by Arthur Edmunds.<sup>1</sup> The kidney-volume changes were measured in an oncometer, smaller in size, but resembling in principle the plethysmograph. Both instruments were connected with piston-recorders arranged to write on a drum just above the pointer of the blood-pressure recorder. The vagi were paralyzed with atropine, and one milligram of lobeline then injected. Both intestinal and renal levers recorded changes that were exactly alike during the primary injection, so one description will fit both. For the first five or eight seconds, both organs underwent an increase in size, reaching the maximum at the end of the time mentioned, which corresponded with the time when the blood-pressure began to rise. With this rise in pressure both curves showed a marked decrease in the volume of the organs, the minimum size being reached at the same time as the maximum blood-pressure was attained. From this time, as the general blood-pressure fell, the volume of the organs increased until they were both markedly congested, compared with their condition before lobeline had been injected. In subsequent injections in which at times larger doses (five milligrams) were given, the same series of events took place in the intestine, that is, with a rise in the blood-pressure was a lessening of intestinal blood-content; but in no case after the primary injection did a diminution in kidney-volume occur, but, on the other hand, its volume increased with the blood-pressure rise.

The work of V. Basch and Oser on nicotine is confirmed by Langley and Dickinson, who described a general constriction of the abdominal vessels, shown by the pallor of the organs; this pallor, after lasting one or two minutes, is followed by flushing. Neither observer records a primary flush coming on before the pallor, and in this they agree with my work on lobeline, as when the intestines are observed under a pane of glass in a warm salt solution bath the first change noted was the pallor. However, when the changes are recorded by a plethysmograph, the primary increase in volume is easily made out.

The changes in the general blood-pressure with both drugs are therefore due to similar causes; the primary fall in pressure, due to the vagus action, succeeded by the great rise, due to constriction of the vessels controlled by the splanchnics, the action no doubt being caused by a stimulation and secondary paralysis of the ganglia on

<sup>1</sup> A. EDMUNDS: *Journal of physiology*, 1898, xxii, p. 380.

the course of the nerves mentioned. The renal ganglia being paralyzed before those which send fibres to the mesenteric vessels, the volume of the kidney may not lessen in injections after the primary, while the intestinal vessels may still undergo constriction.

#### EFFECT ON THE EYE.

Ronnberg found in cats narrowing of the pupil caused by lobeline. Dreser reported in cats, contraction of the pupil taking place in twenty minutes if the drug was applied locally, while he got dilatation upon internal administration.

**Personal observations.**—In a rabbit anæsthetized with paraldehyde, I found after intravenous injections of from three to ten milligrams of lobeline, contraction of the pupil occurred at once. Secondary injections made soon after had no effect.

In cats, anæsthetized by chloretone and morphine or by chloretone alone, intravenous injections of one milligram gave dilatation of the pupil, lasting for a few seconds, followed by contraction.

Extirpation of the superior cervical ganglion in either the cats or rabbits did not alter the results given above, excepting that the pupil, on the side on which the ganglion had been removed, was always smaller than the pupil on the intact side, the dilatation in the case of the cat, and the contraction in the experiments with the rabbits, not being sufficient to overcome the original difference between the two sides. On local application to the eye of the cat, no change whatever in the pupil could be obtained.

After lobeline had been injected, stimulation of the cervical sympathetic below the superior ganglion had no effect on the pupil, while stimulation above caused prompt dilatation, showing that the ganglion was paralyzed by the drug and that the extreme terminations were intact.

Langley and Anderson<sup>1</sup> report paralysis of the endings of the third nerve in the external muscles of the eye of the rabbit by nicotine. On account of the close relationship existing between the action of the two drugs, experiments were made to see if the same effects could be got with lobeline, but no such effects were obtained in either rabbits or cats, using, respectively, doses of fifteen and twenty-five milligrams of the drug.

<sup>1</sup> LANGLEY and ANDERSON: *Journal of physiology*, 1892, xiii, p. 460.

*Experiment. To show the action on the rabbit's pupil, November 4, 1903. —*

White rabbit, 2100 grams. 3.75 c.c. paraldehyde. Right superior cervical ganglion extirpated. The size of pupil measured with scale.

Time.	R.	L.
3.15	8 mm.	9 mm.
3.19	3 mgm. lobeline into jugular vein.	
3.20	6 mm.	6.5 mm.
3.21	5 "	6 "
3.32	5 "	6 "
3.37	6 "	7 "
3.38	5 mgm. lobeline.	
3.40	6 mm.	7 mm.
3.42	6 "	7 "
3.55	6.75 mm.	7.25 mm.

In general it may be said that the action of lobeline on the pupil resembles that of nicotine, when the drugs are given intravenously. Extirpation of the superior cervical ganglion in cats is said to delay, at times, the dilatation of the pupil which is caused by nicotine. This delay of five or ten seconds was not seen under lobeline action.

On local application of nicotine to the eyes of dogs and cats, Langley and Dickinson obtained only dilatation, while Grunhagen stated the drug often causes no constriction, and Rogow found, in cats, once no change, once dilatation, and once dilatation followed by constriction. As given above, lobeline was not seen to have any effect on the cat's pupil, when applied locally.

The differences between the actions of the two drugs on the terminations of the third nerve have been given.

#### ACTION ON THE SALIVARY GLAND.

A large dog was anæsthetized with morphine and chloretone, and the submaxillary duct dissected out, and a cannula inserted into it, the other end of the cannula being connected with a horizontal tube with a scale behind it.

The lingual nerve was also exposed, as well as the chorda tympani, both nerves being cut centrally. The drug was injected into the jugular vein.

*Experiment. —*

Time.	
10.05	Stimulation of chorda tympani. — Active secretion.
10.08	2 mgms. lobeline into jugular. — Secretion began, and fluid in tube moved 10 cm. and then stopped.
10.12	Stimulation of chorda. — No effect.
10.15	2 mgms. lobeline. — No effect.
10.18	5 mgms. pilocarpine. — Profuse secretion.
10.25	1 mgm. atropine. — Secretion stopped.
10.30	5 mgms. pilocarpine. — No effect.

This experiment shows that lobeline first stimulates and then paralyzes the submaxillary ganglion, but has no effect on the extreme terminations of the nerves, as pilocarpine later causes a profuse secretion.

## ACTION ON VARIOUS SYMPATHETIC GANGLIA.

An interesting point encountered in the work on lobeline was the relative ease with which the drug paralyzed the different sympathetic ganglia in the body.

For instance, in cats the superior cervical and the vagus ganglia are paralyzed by very small doses (one milligram). Also, in the same animal, the ganglia on the constrictor fibres distributed to the renal vessels are paralyzed by very nearly the same amounts as will affect the ganglia mentioned above, while it takes a larger dose to paralyze the ganglia on the constrictor fibres which supply the intestinal vessels. This then gives a rise of blood-pressure due to constriction of the mesenteric vessels, after we fail to get narrowing of the renal vessels, or the customary effects from stimulation of the peripheral end of the vagus nerve, or the central end of the cervical sympathetic. Also, with cats and dogs, the accelerator action is present later than the inhibitory, as it takes a larger dose to paralyze the inferior cervical ganglion than it does the vagus ganglion.

Nicotine paralyzes the ciliary ganglion more easily than it does the superior cervical ganglion (Langley and Anderson).

## TOLERANCE.

A study of the comparative action of the two alkaloids, lobeline and nicotine, could not fail to impress one with their great similarity and close relationship as far as their pharmacological action is con-

cerned. For this reason, it was thought that it would be interesting to try and get tolerance in animals to each of the drugs, and then to see if the tolerance gained by an animal to one of the drugs would give it also tolerance to the other. Examples of this tolerance to one drug, giving also a like resistance to closely related drugs, is illustrated, as is well known, in the hypnotics.

For this purpose cats of the same size were chosen, and the drugs in dilute solution given by the mouth upon an empty stomach, nicotine acid tartrate and lobeline hydrochloride being used. As a measure of the degree of tolerance attained, the dose required to cause vomiting was most convenient. In each animal the minimal emetic dose of each drug was ascertained, and then daily injections were made of doses slightly smaller than this. The results obtained were rather surprising. In neither animal could tolerance be obtained. During the thirty days the experiment was carried on, the doses, especially that of nicotine, had to be lessened every three or four days to avoid vomiting. At first fifteen milligrams of nicotine acid tartrate caused no emesis, later the dose had to be reduced to twelve milligrams, then to ten, and finally eight milligrams caused vomiting. Likewise, at first four milligrams of lobeline caused no symptoms beyond salivation, but later, toward the end of the month, a dose of the same size produced marked vomiting every day it was given. The fact that no tolerance could be gained to either drug is rather surprising, especially so when the rapid and easy tolerance gained by men to nicotine is remembered. Also Vas<sup>1</sup> reported getting tolerance to nicotine in rabbits, but as no vomiting occurs in these animals, he did not have as good an index of the degree attained.

#### SUMMARY.

In frogs, lobeline causes some excitation, followed by depression of the central nervous system, with lack of muscular co-ordination, and, later, a total loss of reflexes, and paralysis due to a curara-like action on the motor terminations. If the animal recovers, it occasionally exhibits marked reflex excitability.

On the hearts of cold-blooded animals (frogs and turtles), in addition to a nicotine-like action, lobeline also acts on the heart-muscle directly, and inhibits the actions of such a drug as muscarine. In

<sup>1</sup> VAS: *Archiv für experimentelle Pathologie und Pharmakologie*, 1894, xxxiii, p. 141.

larger doses the heart-muscle is depressed, weakening and slowing the contractions.

In warm-blooded animals (cats and dogs), the main effects of small doses are due to the powerful emetic action of the drug, most of the symptoms being secondary to the vomiting, which is due to action on the medulla. In larger doses muscular twitchings are followed by tonic convulsions and death.

The drug is a powerful respiratory stimulant in small doses, and in large quantities causes death by paralyzing the respiratory centre. Stimulation of the vagus nerve has no effect on the bronchial muscles after lobeline (Dreser).

The effects of the drug upon the rate of the mammalian heart, the height of the blood-pressure, the circulation in the abdominal viscera and in the ear (rabbits), the changes in the pupil and the submaxillary gland may all be explained by a primary stimulation and final paralysis of the various sympathetic ganglia controlling these functions.

Lobeline paralyzes the superior cervical, the vagus, and the renal ganglia more easily than it does the inferior cervical and the mesenteric ganglia.

Tolerance to the drug could not be gained in cats.



# AN EXPERIMENTAL STUDY OF THE RHYTHMIC ACTIVITY OF ISOLATED STRIPS OF THE HEART-MUSCLE.

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THIS study was undertaken at the suggestion and under the direction of Dr. Howell, with the hope of throwing some additional light upon the problem of the interaction of ions, especially of sodium and calcium, in rhythmically contractile tissues.

Strips from the apex of the ventricle were used, prepared as described by Greene.<sup>1</sup> In many experiments four strips from the same heart were used. These were obtained by cutting the usual apex strips crosswise in the middle. The records given by these strips, though small, were in every other respect comparable with those obtained from pieces of tissue of the usual size. The great advantage of this procedure lay in the fact that three controlled experiments could be carried on at once, instead of one, as by the usual method, hence a greater range of comparison was possible. The distilled water and the salts used in the experiments were prepared as described by Greene.<sup>2</sup> The salts used were the chlorides of sodium, calcium, and potassium.

Lingle<sup>3</sup> has stated that the action of ions in initiating spontaneous contractions may be looked upon as entirely distinct from the action of these same ions in maintaining them after they have begun. It has seemed to the author reasonable to suppose that if, as has been suggested by several observers, spontaneous contractions are the sign of a certain relationship existing among the ions in the tissue, then whenever the proper relationship is present, whether naturally or as the result of artificial manipulation, spontaneous contractions will occur, whether or not the tissue has been previously active. In other

<sup>1</sup> GREENE: This journal, 1898, ii, p. 83.

<sup>2</sup> GREENE: *Loc. cit.*, p. 86.

<sup>3</sup> LINGLE: This journal, 1900, iv, p. 270.

words, whatever condition suffices to initiate beats should, if maintained, be sufficient to cause their continuance. In order to test this idea, the experiments described in this connection were carried on. The object immediately in view was the study of every possible method of initiating beats in resting strips, and of every environment under which they continue active after beats have commenced. Such a study is, of necessity, a work requiring very much time for its completion, but some of the results thus far obtained may throw enough light on the problem under consideration to make their presentation at this time worth while.

The experiments here recorded were performed during the year 1903. During the winter and autumn months the work was carried on in this laboratory, and the animal studied was the common slider terrapin, *Pseudemys hieroglyphica*. During the spring and summer the author worked in a temporary laboratory established by himself in Minnesota. The work in this summer laboratory was almost entirely upon the common mud turtle, *Chrysemys marginata*. A few specimens of *Chelydra serpentina* were also studied. It should be stated at the outset that specific differences in the reactions of heart-tissue, if any were present, were neither conspicuous nor constant enough to necessitate differentiation in the study of the experiments. Seasonal variations seemed quite marked in some instances. These will be considered in their proper place. What seems to the author to be the typical reaction is obtained from the heart of an animal in winter condition, studied shortly after it is removed from its natural habitat.

#### THE INITIAL STANDSTILL.

Whenever a strip is cut from a beating ventricle, it immediately comes to rest in diastole. Gaskell<sup>1</sup> states that this standstill is frequently only temporary, and that, after a longer or shorter interval, the strip resumes rhythmic activity spontaneously. Observers in recent years have not been able to obtain this result, except at rare intervals, on strips suspended in air, and left entirely without treatment. We may, therefore, safely conclude that, under normal conditions, the isolated ventricle is not ordinarily spontaneously rhythmical. Lingle<sup>2</sup> attributes the standstill of the isolated ventricular strip to the mechanical shock of the operation by which it

<sup>1</sup> GASKELL: Philosophical transactions, London, 1882, p. 993.

<sup>2</sup> LINGLE: This journal, 1903, viii, p. 76.

is prepared for study. It would seem, however, that if mechanical shock is the only factor concerned in the failure of the strip to beat, spontaneous recovery should be the rule, rather than the very rare exception. An experiment will be cited presently in which a strip, under proper conditions, was isolated completely without disturbance of the continuity of its rhythm, although suffering as much mechanical violence as any strip does in preparation.

Howell<sup>1</sup> has suggested the view that the standstill of the isolated ventricle is due to the presence in the circulating medium of some substance whose influence upon the ventricle is inhibitory. According to his view, the potassium ions play this inhibitory rôle. As is well known, the simplest and, in fact, the usual method of inducing beats in a strip of ventricle, is by immersing the strip in 0.7 per cent sodium chloride solution, in which, if alive, it will invariably begin, sooner or later, to beat rhythmically. Precisely the same result will follow if the solution contain, in addition to sodium chloride, the physiological proportion of calcium chloride,—about 0.025 per cent. If, however, there be present in the solution, in addition to these, the physiological proportion of potassium chloride, about 0.03 per cent, the strip will, in most cases, remain quiet indefinitely. Permanent quiescence can be maintained, in virtually every case, by increasing slightly the proportion of potassium chloride in the solution. Granting that rhythmic activity depends upon ionic interaction, the facts cited above certainly indicate the correctness of Howell's view. The tissue contains sodium, calcium, and potassium ions at the outset; the only one of these whose outward diffusion must be permitted, in order for the strip to become active, is the potassium. The question as to which are the efficient ions in *producing* activity and *maintaining* it, is entirely distinct from the one now under consideration, and will be discussed in its proper place.

Reference was made above to an experiment in which the mechanical violence of preparing the strip did not cause standstill. The experiment was as follows: A heart was removed from the body and the apex of the ventricle partially severed by two cuts parallel with the margin, as shown in Fig. 1. The preparation was then mounted, as shown in the figure, with the partially severed apex in connection with a recording lever. The apex beat throughout the entire process of preparation synchronously with the remainder of the heart, and continued to do so after suspension. One such preparation was sus-

<sup>1</sup> HOWELL: This journal, 1898, ii, p. 79.

pended in moist air for twenty minutes, during which time the beat of the apex was continuous and vigorous. At the end of this time, the apex was isolated by a cut through the connecting bridge of tissue, whereupon it came promptly to rest, and remained perfectly still throughout the experiment, although the great mass of the organ

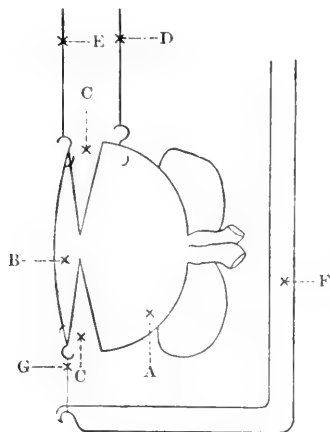


FIGURE 1.—Diagram illustrating the method of suspending a whole heart with partially isolated apex strip. *A*, entire terrapin heart; *B*, apex almost isolated by cuts *C* and *C'*; *D*, thread suspending whole heart to fixed support; *E*, thread connecting apex with recording lever; *F*, glass rod to which apex is attached at lower end by a thread *G*. The whole arranged to be immersed at pleasure in any desired solution.

continued to beat as before. A precisely similar preparation was suspended for twenty minutes in 0.7 per cent sodium chloride solution, after which the apex was isolated in the same way as in the former instance. In this case, however, the isolated apex did not come to rest, but continued to beat regularly, with no pause save a momentary one while the cut was being made. The same amount of mechanical violence was inflicted in each case. The conclusion to be drawn from this experiment is that the standstill of the isolated ventricle is due, not to mechanical violence, but to some factor whose influence is overcome by the treatment with the saline solution. An interesting fact was brought out by this experiment, although bearing upon quite a different problem from the one under consideration. The rhythm of the entire heart, including the partially separated apex, was, in one experiment, twenty-eight per minute. The rhythm of the apex fell, immediately upon isolation, to seven per minute, which rate was maintained for some time. During the later stages of the experiment the rate gradually in-

creased, although the original value was never regained. This experiment indicates that a ventricle in which spontaneous contractions have been artificially induced has a rhythm of its own, which is quite independent of the normal rhythm of the heart as a whole.

## THE LATENT PERIOD.

In a preceding paragraph the idea was advanced that the isolated ventricular strip remains at a standstill for the reason that it is not, under the conditions of normal life, spontaneously rhythmic. It is the purpose of this section to describe the various artificial conditions under which rhythmic contractions may occur.

**Sodium chloride solution.** — The first method of inducing beats has already been mentioned. It consists of immersing the strip in 0.7 per cent sodium chloride solution. A strip in this solution exhibits a period of standstill before the onset of activity. This latent period is of variable length; its average duration, for eighty-five observations made by the author throughout the year, was forty minutes. There appears to be a distinct seasonal variation in this respect. The average latent period for fifty-one observations taken during the months of November, December, January, and February was fifty minutes, while for thirty-one observations during the months of May, June, August, and September the average latent period was twenty-seven minutes. This variation is probably associated with the difference of temperature of the two seasons, although observations which will be reported presently indicate that there is also a definite difference in the condition of the tissue, to which this difference in the latent period may be referred. The extreme range of duration of the latent period was between five and one hundred and thirty-six minutes. Instances of such wide departure from the average are decidedly in the minority, seventy-five per cent of all observations falling reasonably near the average value.

**Preliminary bath containing calcium.** — If a fresh strip be placed for a short time (three to five minutes) in a solution containing 0.7 per cent sodium chloride and 0.025 per cent calcium chloride, and be then transferred to a solution of 0.7 per cent sodium chloride alone, the duration of the latent period is very much less than that shown by strips placed directly in 0.7 per cent sodium chloride without the preliminary treatment with the calcium-containing solution. This result was constant during the winter months, and is so marked that it cannot be regarded as accidental. Table I gives the results of fourteen observations of this fact. The averages from the table are as follows: for those strips which were treated with the solution containing calcium chloride, the average latent period was eight minutes, while for the control strips, which did not receive this

treatment, but were placed directly in 0.7 per cent sodium chloride, the average latent period was thirty-nine minutes. That this effect is due to the calcium of the preliminary solution, is made clear by

TABLE I.

Effect of a brief preliminary bath of a solution containing 0.025 per cent calcium chloride, and 0.7 per cent sodium chloride on the latent period of fresh strips in 0.7 per cent sodium chloride.

Date of observation. 1903.	Latent period of control in 0.7 % NaCl.	Latent period of test strip.	Date of observation. 1903.	Latent period of control in 0.7 % NaCl.	Latent period of test strip.
	minutes	minutes		minutes	minutes
Jan. 23	44	9.5	Feb. 18	..	6.5
" 27	..	5.0	" 18	..	7.0
" 27	..	7.0	" 27	112	13.0
" 28	24	4.0	" 28	35	7.0
" 28	24	11.0	Mar. 2	24	13.0
" 29	10	5.0	" 2	21	10.0
Feb. 5	42	10.0	" 3	51	10.0

TABLE II.

Effect of a brief preliminary bath of 0.025 per cent calcium chloride in sugar solution on the latent period of fresh strips in 0.7 per cent sodium chloride.

Date of observation, 1903.	Latent period of control in 0.7 % NaCl.	Latent period of test strip.
	minutes	minutes
Jan. 24	..	6.0
" 26	56	5.0
" 26	..	9.5
Dec. 15	50	15.0
" 17	70	10.0

the observations recorded in Table II, in which exactly the same proportion of calcium was used as in the former series of observations, but the solution was made isotonic with the serum by means of cane sugar instead of sodium chloride. The average latent period of the

calcium-treated strips in this series of five observations was nine minutes, the control strips had an average latent period of fifty-nine minutes. Greene<sup>1</sup> observed a similar shortening of the latent period when a strong solution of calcium chloride in distilled water was used as a preliminary bath, followed by 0.7 per cent sodium chloride solution. Table III shows that the presence of potassium in small

TABLE III.

Effect of a brief preliminary bath of normal Ringer's solution on the latent period of fresh strips in 0.7 per cent NaCl.

Date of observation. 1903.	Latent period of control in 0.7% NaCl.	Latent period of test strip.
	minutes	minutes
Jan. 7	..	5.0
" 14	..	5.0
" 15	..	3.0
" 20	..	5.5
" 21	22.5	7.5
" 22	60.0	8.0
Feb. 4	..	3.0
Dec. 21	60.0	10.0

amounts in the preliminary solution does not interfere with the action of the calcium. The modification of Ringer's solution described by Howell<sup>2</sup> was used as the preliminary bath in this series of experiments. The average latent period of the calcium-treated strips in this series of eight observations was six minutes. The earlier observations of this series were not controlled; the average latent period of the three controls which were made later, was forty-seven minutes. Several experiments were performed in which small proportions of potassium chloride, from 0.03 per cent to 0.04 per cent were used with 0.7 per cent sodium chloride in the preliminary bath. The presence of the potassium compound did not tend to produce any shortening of the latent period, so far as could be determined from the few tests that were made. If all the observations of the effect of physiological proportions of calcium chloride in the

<sup>1</sup> GREENE: *Loc. cit.* p. 101.

<sup>2</sup> HOWELL: This journal, 1898, ii, p. 53.

preliminary bath be grouped together, the following values are obtained: twenty-seven calcium-treated strips showed an average latent period of 7.5 minutes. Their controls, which had precisely similar treatment, except that they had not the preliminary immersion in a calcium-containing solution, showed an average latent period of forty-four minutes. These observations seem to establish the fact that calcium, under the conditions quoted, has the power to induce contractions in freshly isolated ventricular strips, immersed in 0.7 per cent sodium chloride solution, much more promptly than sodium chloride used alone.

A number of observations, twenty-seven in all, were made in regard to the effect of larger proportions of calcium chloride in the preliminary bath. Solutions were used in which the proportion of calcium chloride ranged from twice to four times the physiological, *i. e.*, from 0.05 per cent to 0.1 per cent. In these experiments the calcium chloride, in eleven cases, caused distinct shortening of the latent period, while in sixteen experiments this was either distinctly lengthened, or was only slightly influenced. The results of this series of experiments are given in Table IV. A careful study of this table brings out a striking fact, which can be made clear by a statement of the average values of the different sets of latent periods. For the eleven cases in which the large dose of calcium shortened the latent period, its average duration was fifteen minutes; the average latent period for the eleven controls was thirty-eight minutes. For the sixteen cases in which calcium delayed the onset of activity, the average latent period was thirty-six minutes; the sixteen controls for these, however, showed an average latent period of only twenty-two minutes. With the exception of three observations, all those in which the large proportion of calcium chloride delayed the onset of activity were made during the summer months, when, as was noted above, the latent period of fresh strips in 0.7 per cent sodium chloride was considerably shorter than in cold weather. The point upon which it is particularly desired to lay stress is this. *The shortening of the latent period in sodium chloride observed during warm weather depends upon the same condition of the tissue as does the delayed activity which calcium sometimes causes.* A study of the average latent periods of the author's summer experiments makes this fact apparent. Of the thirty-one observations of the latent period made during warm weather, thirteen showed an inhibitory action on the part of calcium, eleven showed the opposite effect, while the point



TABLE IV.

Effect of large doses of calcium chloride in the preliminary bath, on the latent period of fresh strips in 0.7 per cent NaCl.

Date of observation. 1903.	Composition of solution.	Latent period of control in 0.7 % NaCl.	Latent period of test strip.
		minutes	minutes
Aug. 29	0.05 % $\text{CaCl}_2$ and 0.7 % NaCl.	65	17
Sept. 3	" " " " "	25	6
Dec. 17	" " " " "	45	10
" 19	" " " " "	25	8
May 6	0.06 " " " "	62	29
" 21	" " " " "	22	7
June 4	" " " " "	21	14
May 7	0.01 % " " " "	30	18
" 8	" " " " "	58	26
Aug. 20	" " " " "	36	17
" 27	" " " " "	36	16
May 15	0.04 % " " " "	5	10
Aug. 5	" " " " "	10	14
" 11	" " " " "	23	22
" 19	0.05 % " " " "	10	24
" 31	" " " " "	40	35
Sept. 5	" " " " "	28	37
" 7	" " " " "	13	16
" 11	" " " " "	5	10
" 12	" " " " "	11	27
Nov. 28	" " " " "	70	92
Dec. 4	" " " " "	35	50
" 10	" " " " "	45	75
May 23	0.06 % " " " "	13	70
" 26	" " " " "	35	80
Aug. 10	0.01 % " " " "	8	10
Sept. 2	" " " " "	4	11

was not determined for the remaining seven. The average latent period for the control strips in sodium chloride, of the thirteen whose test strips showed calcium inhibition, was sixteen minutes, while for the remaining eighteen, the average latent period was thirty-five minutes. The possible significance of these facts will be discussed in a later paragraph.

The facts presented above may be summarized in the following statement of the effect of a preliminary bath of calcium chloride on fresh ventricular strips. This substance in physiological proportion applied to a strip for a short time diminishes markedly the latent period of the strip in 0.7 per cent sodium chloride. In twice the physiological proportion or more, calcium chloride may have the same effect as in physiological concentration upon strips whose condition is typical, but upon strips in an atypical condition, as indicated by a marked shortening of the normal latent period in 0.7 per cent sodium chloride, such concentrations of calcium chloride are likely to exert a distinctly inhibitory influence.

**Continued application of calcium-containing solutions.**—The effects of calcium which have been thus far described were all obtained by treating strips for a short time with a solution containing calcium ions, and then transferring them to a solution of 0.7 per cent sodium chloride in which there were no calcium ions except those that might have been carried over on the tissue. That the effects observed were due to the brief contact with the calcium-containing solution, rather than to the calcium ions which were carried over when the strip was transferred to the pure sodium chloride solution, was shown by the fact that thorough rinsing with several changes of saline solution, or, for that matter, immersing the strip in a flowing stream of 0.7 per cent sodium chloride solution, did not change the effect of the previous application of calcium chloride.

A few experiments, ten in all, were performed in order to see whether the calcium effect would be the same when the strip was immersed in a solution containing both sodium and calcium chlorides, and left there till beats began. Concentrations of calcium chloride ranging from the physiological, 0.025 per cent, to three times that amount, were used. The results were very conflicting. In four experiments the presence of calcium was without effect on the latent period; in four the latent period was distinctly lengthened; and in the two remaining experiments the latent period was markedly shortened. Two of the experiments in which calcium had no effect on the latent

period were performed in the month of May; all the others of this series were carried on in November and December. It seems scarcely reasonable to suppose that the effect of a brief preliminary bath of a calcium-containing solution should be markedly different from that of the same solution applied for a longer time; therefore a more exhaustive study of this latter point is desirable.

**Mixed solutions of sodium chloride and cane sugar.**—Lingle<sup>1</sup> has shown that heart strips may beat spontaneously in mixtures of 0.7 per cent sodium chloride and isotonic cane sugar solution in which the dilution of the sodium chloride is very considerable. The author studied the latent period of strips in such solutions. In the paper just referred to, Lingle<sup>2</sup> notes the fact that the latent period in such dilute solutions is longer than in physiological salt solution.

Two dilutions of sodium chloride with sugar solution were studied by the author. In one the proportion was 10 c.c. 0.7 per cent sodium chloride to 15 c.c. isotonic sugar solution; in the other the proportion was 5 c.c. 0.7 per cent sodium chloride to 20 c.c. of the sugar solution. There were no differences between the latent periods of these two sets of observations that might not fall within the range of individual variation; therefore the entire group is studied together. The average latent period of the control strips in 0.7 per cent sodium chloride, for the entire series of fourteen observations, was fifty-seven minutes. The average latent period of the strips bathed in solutions of sodium chloride diluted with sugar was one hundred and seventy minutes. It should be noted in passing that occasionally a strip of ventricle is observed which remains quiet indefinitely in a solution in which the proportion of sodium chloride is much below the physiological. This occurs more frequently, as Lingle points out, the more the sodium chloride is diluted.

**Long separation from the body.**—A number of observers have called attention to the fact that perfectly fresh heart-tissue is more resistant to interchanges among its salts than the same tissue is after it has been separated from its normal connections for some time. This observation is corroborated by the results of two series of experiments performed by the author. The first series consisted in preparing strips according to the usual method, and suspending them directly in moist air. After hanging thus for some time, each strip was immersed in 0.7 per cent sodium chloride and the latent period

<sup>1</sup> LINGLE: This journal, 1902, viii, p. 91.

<sup>2</sup> LINGLE: *Loc. cit.* p. 91.

in the solution under this condition was compared with that of a perfectly fresh strip in the same solution. The average values for twelve experiments, in which the time of suspension in the moist chamber ranged from one-half to four and one-half hours, are as follows: the latent period of the control strips, placed directly upon isolation into 0.7 per cent sodium chloride, averaged forty-eight minutes; the latent period of the test strips, transferred to 0.7 per cent sodium chloride after long suspension in moist air, averaged fourteen minutes. In the second series the fresh strips were placed in solutions containing 0.7 per cent sodium chloride together with 0.04 per cent potassium chloride. This proportion of potassium chloride is one-fourth larger than the physiological, reckoning the latter to be that used in Ringer's solution, and usually suffices to prevent spontaneous contractions indefinitely. After immersion in this solution from one to three hours, the strips were transferred to 0.7 per cent sodium chloride, and the latent period after transfer compared with that of perfectly fresh strips as before. The results for this series of four experiments are these: the latent period of the strips transferred from the potassium-containing solution to that of sodium chloride alone, averaged in the latter solution twelve minutes. The controls showed an average latent period of fifty-five minutes. In every case the test strip was left in the potassium-containing solution for some time after its companion strip in pure sodium chloride had commenced to beat. In no case was there any sign of activity in the strip, so long as it remained in the solution containing potassium chloride, although it began to beat soon after transfer to a solution of sodium chloride without potassium ions in it. These facts certainly indicate that potassium exerts an inhibitory influence upon the tissue, which is removed by allowing diffusion of potassium to take place. If, as Howell has suggested, the only factor concerned in the normal latent period of fresh strips in sodium chloride is the presence of excess of potassium in the tissue, these experiments also show that diffusion is less rapid in fresh strips than in those isolated for some time.

**Solution of sugar isotonic with the blood.**—Lingle<sup>1</sup> states that strips which have been suspended in a solution of cane sugar, isotonic with the blood, for some time, frequently begin to beat very promptly upon removal to a solution of 0.7 per cent sodium chloride. The author has been unable to obtain this result from strips which were placed in the sugar solution immediately upon isolation, and kept

<sup>1</sup> LINGLE: This journal, 1900, iv, p. 270.

there one hour or more. Such strips have invariably gone into a condition of strong tone, and the first effect of removal to a salt solution has always been a prompt and marked fall of tone, followed ultimately, but only after a number of minutes, in one case one hour, by spontaneous contractions. It should be stated, however, that if the sugar solution contained a small proportion of sodium chloride, not enough to bring about activity itself, transfer to 0.7 per cent sodium chloride was usually followed by contractions very promptly indeed; unless the strip had been too long in the sugar solution, in which event no contractions resulted from the application of the sodium chloride.

**Organic calcium-containing solutions, including blood.**— In connection with the study of the influence of calcium in shortening the

TABLE V.

Effect of a brief preliminary bath of terrapin serum upon the latent period of a fresh strip of terrapin ventricle in 0.7 per cent NaCl.

Date of observation. 1903.	Latent period of control in 0.7 % NaCl.	Latent period of test strip.
	minutes	minutes
Feb. 3	70	15.0
" 4	..	4.5
" 20	27	12.0
" 28	35	15.0
Sept. 3	26	5.0

latent period of fresh strips in 0.7 per cent sodium chloride solution, a point of considerable interest was observed which should be recorded in this connection. After the effect of calcium in inorganic combination, as represented by calcium chloride, had been satisfactorily determined, the question arose as to whether calcium in organic combination would have the same effect. Milk and solutions of calcium glycono-phosphate in sodium chloride solution were tried and found to produce a definite shortening of the latent period. But the serum of the turtle contains calcium in organic or inorganic combination, and ought, therefore, to behave in this respect like other calcium-containing solutions. Serum was obtained by defibrinating the blood of a terrapin, and allowing the corpuscles to settle. A fresh strip was given a preliminary bath for three minutes in twelve cubic

centimetres of this serum. The results of these experiments are given in Table V. The average latent period for the control strips was forty minutes; for the test strips, ten minutes. In a single instance the fresh blood was left at a low temperature for twenty-four hours, and the clear, unclotted plasma thus obtained was tested. During the three minutes that this plasma was about the strip, clotting began in it. It reduced the latent period of the test strip to thirteen minutes, while the control strip had a latent period of thirty-four minutes. Fresh blood itself was drawn, and used immediately as a preliminary bath about the fresh strip. Frequently it could be

TABLE VI.

Effect of a brief preliminary bath of fresh terrapin blood upon the latent period of a fresh strip of terrapin ventricle in 0.7 per cent NaCl.

Date of observation. 1903.	Latent period of control in 0.7 % NaCl.	Latent period of test strip.
Part 1.—Blood clotted on the strip.		
	minutes	minutes
Feb. 11	26	16
" 21	30	15
" 28	35	10
Part 2.—Blood did not clot on the strip.		
Feb. 9	45	72
" 11	40	39
" 12	41	56

kept about the strip for three minutes, and then be withdrawn without clotting. In every case in which this was done, and the blood adhering to the strip was removed with care by thorough rinsing, no shortening of the latent period was observed. On the other hand, if the blood were allowed to clot about the strip before being removed, the latent period was shortened in the same way as when serum was used. The experiments with blood are recorded in Table VI. The average latent period for nine observations with serum, clotted plasma, and clotted blood was, for the test strips, twelve minutes, and for the controls, thirty-six minutes. These experiments indicate clearly that

the calcium in the normal uncoagulated blood is in a condition different from that in which it exists in the serum after coagulation. In the latter state it behaves, so far as the relationship that we are now studying is concerned, like the aqueous solution of a calcium salt, and it is possible, therefore, that the difference here pointed out between the calcium of the fresh plasma and of the serum may be connected with the degree of ionization that it undergoes in the two conditions.

**Summary of the important conditions affecting the latent period.—**

1. A mixture of 0.7 per cent sodium chloride with the proper proportion of calcium chloride, followed in a few minutes by 0.7 per cent sodium chloride alone, induces spontaneous beats in a freshly isolated ventricular strip more promptly than any other means now known; the average time is about ten minutes.

2. Spontaneous beats begin in a fresh strip in 0.7 per cent sodium chloride after a latent period four to five times as long as in Case 1.

3. Spontaneous beats begin in a fresh strip in sodium chloride diluted with isotonic sugar solution after a latent period three times as long as in Case 2.

4. Under certain conditions of the tissue, the latent period of fresh strips in 0.7 per cent sodium chloride solution is less than half the normal. Under these same conditions calcium, in slightly excessive doses, may exert an inhibitory rather than an accelerating influence.

5. The calcium-content of normal, uncoagulated plasma is in such a form that it does not exert the stimulating influence of inorganic calcium salts in solution upon freshly isolated ventricular strips, but during the process of clotting the calcium of the serum becomes efficient in this respect.

#### THE REACTIONS OF STRIPS IN 0.7 PER CENT SODIUM CHLORIDE SOLUTION.

The first effect of placing a freshly prepared ventricular strip in 0.7 per cent sodium chloride is a marked loss of tone. At the outset this is probably merely the normal relaxation from the condition of excessive tone into which the process of preparation has thrown the tissue. This large initial fall is succeeded by a gradual but continuous fall which continues till the death of the strip, if it be left in the sodium chloride solution that long.

Spontaneous beats begin in the strip after a latent period which has been sufficiently described in preceding paragraphs. The series in 0.7 per cent sodium chloride has a perfectly characteristic form, which has been fully described by Greene.<sup>1</sup> In brief it is as follows: The contractions may or may not show a distinct "Treppe," but after the maximum height is reached there is invariably a gradual continuous decline in the extent of contraction, terminating in a condition of practical standstill, which is known commonly as sodium chloride exhaustion. The duration of the series in 0.7 per cent sodium chloride shows wide variation. In the author's experiments it ranged from one-half hour to four hours; the average duration for sixty-eight observations was one hour, fifty minutes. In determining the length of these series, the strip was considered at rest when its actual shortening was less than 0.1 mm. Undoubtedly minute contractions continued for some time after that point was reached, which would make the average given above too small; for the purpose of this study, however, the figure given is sufficiently accurate. The extent of shortening of a strip in 0.7 per cent sodium chloride corresponds, at its maximum, about with that shown by fresh strips in air when stimulated electrically. It is by no means the utmost which the strip can be made to show under proper conditions.

As has been proved repeatedly, complete sodium chloride exhaustion does not imply that the tissue has suffered permanent injury, inasmuch as it may be revived by suitable means, and kept in vigorous activity for many hours. Continued exposure to the sodium chloride solution, after the exhaustion has become complete, does result, however, in the death of the tissue in a comparatively short time. Three distinct methods of restoring the activity of a strip which has come to rest in 0.7 per cent sodium chloride are known to the author.<sup>2</sup> These are: (1) the addition of a small proportion of calcium chloride to the solution in which exhaustion has taken place (the concentration of calcium chloride usually employed varies between 0.025 per cent and 0.05 per cent); (2) the transfer of the strip to an isotonic cane sugar, or similar "indifferent," solution; (3) removal of the strip to a moist air chamber.

The manner of recovery by each of these methods is quite characteristic of the method, and the variation undoubtedly depends upon the different changes which are brought about in the tissue as the

<sup>1</sup> GREENE: *Loc. cit.*, p. 91 *et seq.*

<sup>2</sup> HOWELL: *Loc. cit.*, pp. 185, 189; LINGLE: This journal, 1902, viii, p. 83.



result of the different environments, all leading to the same result, *i. e.*, renewed vigor of contraction. The effect of adding calcium chloride to the solution of 0.7 per cent sodium chloride, in which the strip has come to rest, is a very prompt and marked increase of vigor. The strip usually shows an increased beat in the very first contraction it makes after the addition of the calcium chloride, while within a half-dozen contractions the size of the beat will be considerable; sometimes nearly as great as at the beginning of the sodium chloride series. There is usually a prompt rise in tone as the result of the addition of calcium chloride; where the amount of the calcium salt is relatively large, this tonic shortening may be considerable. When a strip, exhausted in sodium chloride, is transferred to an isotonic sugar solution, the effect is more gradual, although it usually begins to develop within a short time after the application of the sugar solution. The increase in beat is slow but continuous, reaching its maximum about one half-hour after the first indication is given. Sometimes there is a slight rise of tone in the sugar solution, but not usually. The reviving effect of placing the exhausted strip in a moist chamber becomes apparent after a much longer interval than by either of the other methods described. In only one of the author's experiments could any change be detected in less than ten minutes, while in several instances an hour elapsed before the least increase in vigor could be seen. Once established, however, the increase in beat is continuous till the maximum is reached. This may be in from two and one-half to ten hours; the average of fifteen observations was about five hours. The ultimate vigor attained by strips, revived in moist chambers, usually equals the maximum exhibited by them in 0.7 per cent sodium chloride, and frequently exceeds that value.

The revival brought about by the addition of calcium chloride to the exhausting solution must be considered as due directly to the presence of calcium, since in no other respect has the solution been altered. The amount of calcium chloride which must be added to produce revival is too small to cause any measurable change in the osmotic relations of the solution, or in the proportion of chlorine ions in it. The revival which results from placing the strip in sugar solution may be explained as a direct effect of the sugar, or else as due to the diffusion of all salts, sodium chloride with the rest, out of the tissue. Sugar, as a non-electrolyte, is generally considered to be without specific action of the sort under discussion. If this assump-

tion be allowed, the revival in sugar is to be traced, perhaps, to the free diffusion of all salts which it permits. The revival in moist air would seem to be due to the direct application of the oxygen of the air to the tissue, or else to be related to the fact that this treatment brings to an end all possibility of either inward or outward diffusion. Lingle<sup>1</sup> suggests that the strip in sodium chloride exhaustion is in a state of mild asphyxia which is relieved by the transfer to air. Experiments will be cited presently, in another connection, in which strips beat forcibly and continuously for very many hours in other solutions whose volume was exactly the same as that of a 0.7 per cent sodium chloride solution which produced exhaustion in two hours. Moreover, the experiment of immersing the strip in a rapidly flowing stream of 0.7 per cent sodium chloride was carried out by the author, with no apparent change in the onset of exhaustion. These experiments make it very improbable that lack of oxygen has anything to do with sodium chloride arrest, or that supplying oxygen freely is the cause of revival in moist chambers. Lingle<sup>2</sup> lays great stress upon the remarkable revival which results from placing an exhausted strip in an atmosphere of pure oxygen. This revival differs, however, not at all in kind, but only in degree from that seen in ordinary air. That oxygen exerts an influence upon the metabolism which lies at the basis of all contractions, can be readily granted. That the mere presence of oxygen is sufficient of itself to cause contractions in the quiescent strip is yet to be proven. Lingle's attempt to prove it<sup>3</sup> by adding hydrogen peroxide to the exhausting solution is insufficient, because the strip, under these conditions, becomes completely enclosed in a layer of bubbles; it is in effect removed from the solution, entirely so, so far as diffusion relations are concerned. The experiment becomes, then, merely another instance of revival by suspension in a moist chamber. In a later paragraph an attempt will be made to show that the revival which occurs in moist air can be satisfactorily explained upon the other alternative which was suggested above, *i. e.*, the elimination of all possibility of diffusion.

<sup>1</sup> LINGLE: *Loc. cit.*, p. 98.

<sup>2</sup> LINGLE: *Loc. cit.*, p. 83.

<sup>3</sup> LINGLE: *Loc. cit.*, p. 81.

## THE REACTIONS OF STRIPS IN MIXTURES OF 0.7 PER CENT SODIUM CHLORIDE WITH ISOTONIC SUGAR SOLUTION.

After the initial relaxation, which, as noted in a previous paragraph, is the first phenomenon exhibited by a freshly prepared strip of ventricle after suspension, the tone changes shown by strips in mixtures of sodium chloride and sugar solution depend upon the relative concentration of the two substances in solution. Where the proportion of sodium chloride is rather large the tone falls continuously, as it does in pure salt solution. With the sodium chloride quite dilute, the relaxation may come to an end soon after the strip resumes what may be called its normal length. The author has never observed any increase in tone, so long as some sodium chloride was in the solution, although it occurs constantly in pure sugar solution.

Sufficient mention has already been made of the fact that in the solutions under discussion the latent period is always much longer than in solutions of 0.7 per cent sodium chloride. The series of contractions given by strips in these mixed solutions of sugar and sodium chloride show some points of interest. In the first place, the extent of the initial beats of the series is always much greater than in any other environment with which the author has worked. Under favorable circumstances these initial contractions in dilute salt solution have been almost twice as high as those given by the same strip under electrical stimulation, when freshly isolated and hanging in the air. This great initial vigor is so marked that it cannot escape attention, and should be taken into account in considering the conditions of the heart's activity. The general form of curve given by strips in salt-sugar solutions is similar to that of strips in pure sodium chloride solution. The series, however, is always much longer; in the author's experiments the average duration of series in solutions of sugar mixed with salt was eight and one-half hours, compared with about two hours for the controls in 0.7 per cent sodium chloride. The longer duration is compensated largely by the slower rate of contraction. In one experiment the control in 0.7 per cent sodium chloride gave one thousand contractions in two hours, at the end of which time it was completely exhausted; the companion strip, in sodium chloride mixed with sugar solution, had given thirteen hundred contractions in eight hours when the drum stopped, so that the rest of the record was lost. At the end of the eight hours the strip was by

no means exhausted, although its contractions were quite small. The series usually do not show the regularity of sequence that is so characteristic of the beat in pure sodium chloride. In minor details they may exhibit various modifications. Exhaustion is sometimes accompanied by a gradual decline of the vigor of contraction to zero; in other cases the strip stops beating while the force of the individual beats is considerable. Recovery after a strip had come to rest in a sugar-salt solution was obtained by the author in three ways: (1) by transferring the strip to 0.7 per cent sodium chloride. In the two experiments in which recovery was obtained in this way it was prompt in showing itself, the first beat was more vigorous than any that succeeded it, though by no means so strong as those given by the same strip in the early part of the series in the mixed solution, and the length of the series in the pure salt solution was about the average for this solution. The form of curve was typical for 0.7 per cent sodium chloride, save for some irregularity of rate. (2) By transferring the strip to a moist chamber. The revival by this means was exactly similar, in the two cases in which it was tried, to that shown under the same environment by strips exhausted in 0.7 per cent sodium chloride. (3) By transferring the exhausted strip to a solution containing 0.7 per cent sodium chloride and 0.025 per cent calcium chloride. In two cases the revival by this means resembled that brought about by pure sodium chloride, in that the first contraction after recovery was greater than any succeeding ones, although in other respects there was no marked similarity. In two other cases there was a gradual increase of beat from zero to the maximum. In three of the four cases there was marked rise in tone.

THE REACTIONS OF STRIPS IN SOLUTIONS CONTAINING, IN ADDITION  
TO 0.7 PER CENT SODIUM CHLORIDE, FROM 0.025 PER CENT TO  
0.06 PER CENT CALCIUM CHLORIDE.

Fresh strips placed in solutions containing sodium and calcium chlorides in the proportions given above, show no loss of tone other than the normal initial relaxation. In nearly every instance a rise in tone comes on after the strip has been in the solution for some time. If the proportion of calcium chloride is sufficiently great, this tonic contraction may become excessive, the condition being described by Howell<sup>1</sup> as calcium rigor. The series of spontaneous contractions

<sup>1</sup> HOWELL: This journal, 1902, vi, p. 187.

given by strips in these sodium-calcium solutions differ chiefly from those in pure sodium chloride solution in their longer duration. The range of length of series in the author's experiments was from four and one-half to forty and one-half hours; the average duration of twelve series was seventeen hours. When strips which have been exhausted in 0.7 per cent sodium chloride solution are revived by the addition of calcium chloride to the solution, the series which result are essentially similar to those given by fresh strips in solutions of sodium and calcium chlorides. The average duration of five such series was twenty hours. Strips which have been exhausted, first in 0.7 per cent sodium chloride, and then in isotonic cane sugar, and have then been placed in a sodium-calcium solution, show in this solution the same sort of long-continued series as in the same solution after other preliminary treatment, such as is described above. A noteworthy fact in regard to the effect of solutions of sodium and calcium chloride upon strips, is that when a strip has come to rest in such a mixed solution, it appears to be in a condition from which it cannot be recovered. Neither transfer to pure 0.7 per cent sodium chloride, nor to isotonic cane sugar, nor to moist air has any reviving effect, although, as will be shown, no other kind of exhaustion studied by the author failed to be overcome by one or the other of these methods.

#### THE REACTIONS OF STRIPS IN NORMAL RINGER'S SOLUTION.

The statement was made in a previous paragraph that fresh strips, placed in Ringer's solution, do not usually show spontaneous contractions. Occasionally, however, they may do so. The author observed this result three times in the course of his experiments. Each of these occurred during warm weather. The contractions were vigorous but infrequent; the average duration of the series was six hours. In this, as in every observation of series of contractions in Normal Ringer, the end of the series was marked by increasing length of interval between contractions, without any marked diminution in the extent of the individual beats. Cessation of activity seemed more like the result of an inhibition than of such exhaustion as occurs in 0.7 per cent sodium chloride.

When a strip, after exhaustion in sodium chloride solution, is placed in Ringer's fluid, the first effect corresponds exactly with that observed when revival is brought about by the addition of calcium

chloride to the exhausting solution; there is a prompt increase of extent of contraction. After a short time, however, the rhythm becomes irregular and the beats infrequent, as compared with series in solutions containing only sodium and calcium chlorides, without potassium chloride. A Ringer series after sodium chloride exhaustion is shorter than a corresponding series in a sodium-calcium solution. The average length of series in Ringer's fluid, after sodium chloride exhaustion, was, for five observations, twelve hours; against twenty hours for series in a sodium-calcium solution under corresponding conditions.

Transfer to Ringer's solution after successive exhaustion in 0.7 per cent sodium chloride and isotonic sugar solution, is followed by essentially the same activity as after sodium chloride alone. In the author's experiments the average length of the series after this double exhaustion was four hours more than when the transfer was made directly after sodium chloride exhaustion. Strips which have come to rest in Ringer's solution may be revived with the greatest promptness by transferring them to 0.7 per cent sodium chloride solution, or to a mixture of that solution with calcium chloride in physiological concentration. These results were obtained by the author with strips which had been active in Ringer's fluid for forty hours. The extent of contraction after revival is the same as that shown in the final beats executed by the strip in the Ringer, and these latter may equal the most vigorous beats given in any part of the series, or they may be somewhat less powerful. These facts seem to indicate strongly that cessation of activity in Ringer's solution is a potassium effect.

#### THE REACTIONS OF STRIPS IN A MOIST CHAMBER.

Freshly isolated strips, as has been shown, do not usually exhibit spontaneous activity if suspended directly in moist air; if, however, the strip be subjected to the proper treatment before being placed in the moist chamber, very long and excellent series of contractions may be obtained. If a strip be suspended in 0.7 per cent sodium chloride, or in a sodium-calcium solution, until it executes a single contraction, and be then quickly transferred to a moist chamber, a series of beats is obtained similar to those given by strips in sodium chloride solution in that the force of contraction declines continuously from the beginning, but differing from the series in such solutions in being considerably longer, and also by the fact that the contractions become

more and more infrequent toward the end of the series. The greatest length of series observed by the author under these conditions was fourteen and a half hours; the average for seventeen observations was four hours. Strips which have been placed, thus, in moist air at the very outset of their activity, and have proceeded to exhaustion in the moist chamber, may be recovered well by being returned to the solution in which activity was first induced. An interesting point about the recovery that is brought about by the use of 0.7 per cent sodium chloride is that in several cases, under the conditions now being considered, it began with feeble contractions which improved gradually till the maximum was attained. In every other instance in which the author observed either the initiation or the strengthening of beats by means of sodium chloride, the very first contraction after the application of the salt solution was either maximal or slightly submaximal. Recovery was also obtained by placing the strip in a solution containing 0.05 per cent calcium chloride in 0.7 per cent sodium chloride, and in a single instance by the momentary application of a solution of 0.05 per cent calcium chloride in sugar solution, isotonic with 0.7 per cent sodium chloride.

Reference has already been made to the excellent series of contractions that can be obtained by removing strips which have been completely exhausted in 0.7 per cent sodium chloride to moist air. These series ranged, in the author's experiments, from seven to twenty-seven hours in length; the average for thirteen observations was sixteen hours. The maximal force of contraction, which was reached four to eight hours after the transfer to the moist chamber, was frequently as great as that shown by the strip at the beginning of the sodium chloride series. For the first few hours after being placed in the moist chamber the rate of beat was rapid and regular. In the later stages of the series, however, it became slow and quite irregular. The appearance of the tracing taken during the latter part of a moist chamber series resembles very closely that given by a strip beating in Ringer's solution. The end of the moist chamber series was always marked by the long intervals between the final contractions. Frequently there was also a gradual decline in the force of the contractions during the later stages of the series, but in a number of instances the only indication of exhaustion was the growing infrequency of the contractions. Strips which have come to rest after hours of activity in moist air may be revived in various ways. One of these is by returning the strip to 0.7 per cent sodium chloride.

In five experiments, in which the duration of the series in the moist chamber varied between sixteen and twenty-seven hours, recovery was obtained in this way. In two cases the strip had come to rest after a period of lessening frequency without any diminution in the force of the beats. In both these cases the vigor after revival was as great as at the end of the moist chamber series. In three cases the force of contraction had declined gradually to zero before the transfer to 0.7 per cent sodium chloride. In these cases the maximum vigor after revival was markedly less than the greatest previously shown by the strip. In every instance the series in the salt solution was typical of the solution; in other words, there was a continuous decline in the extent of the contractions. The length of the series varied from twelve to fifty-five minutes; the average for the five observations was twenty-eight minutes. Another method of reviving a strip after exhaustion in a moist chamber, is by treating it with mixtures of calcium and sodium chlorides in solution, using the same proportion of calcium chloride as in the other experiments reported. The recovery by this means is characterized by the vigor of the resulting contractions, which is much greater than that shown by strips recovered by sodium chloride alone, by the long duration of the series after recovery, by the rise in tone which usually occurs, and by the fact that recovery ensues very promptly after the application of the calcium chloride to the strip.

A fact of interest in connection with the activity of ventricular tissue in moist air, is the remarkable stimulating influence which is exerted by solutions containing calcium, when such solutions are allowed to drip over the surface of the tissue.<sup>1</sup> The author used for this purpose solutions of calcium chloride in sodium chloride solution; of calcium chloride in isotonic sugar solution; and specimens of ordinary well-water, which showed a strong calcium test with ammonium oxalate. The method of application was as follows: a few drops were forced from a pipette upon the silk thread connecting the muscle with the recording lever, whence they ran down over the surface of the tissue. The reviving effect was observed repeatedly, and at various stages of the moist chamber series. If the strip was in a state of exhaustion, either after sodium chloride or after a long period of activity in a moist chamber, the effect corresponded with that shown when a bath containing calcium is applied to the strip;

<sup>1</sup> GREENE: *Loc. cit.*, p. 107.



the extent of contraction was promptly and markedly increased. If the strip was beating with vigor, but at long intervals, the effect of moistening it with the calcium-containing solution was to improve the rate and rhythm. That this is a calcium effect solely is indicated by the fact that the revival caused by calcium chloride in sugar solution, or by the calcium-containing water, could not be distinguished from that which resulted from the use of mixtures of calcium and sodium chlorides; moreover, similar moistening with 0.7 per cent sodium chloride alone was absolutely without effect, although it was tried repeatedly in circumstances in which moistening with a calcium-containing solution, or even *immersion* in a solution of sodium chloride, would almost certainly have resulted in recovery. Although the application of calcium is transient, the effect is usually prolonged. In a number of instances in the author's experience a strip thus revived showed the beneficial effect of the treatment for five or six hours after its application. It should be borne in mind, in this connection, that while these calcium effects may result from a very momentary action on the part of the calcium-containing solution, initiation of beats or revival of vigor brought about by sodium chloride alone, invariably requires that the strip be immersed in the sodium chloride solution.

A number of observations were made on the effect of placing a fresh strip in a moist chamber after a brief immersion in 0.7 per cent sodium chloride, or the same solution containing also 0.05 per cent calcium chloride. The latter solution was left about the strip for five minutes in every case; the former was left for variable times up to fifteen minutes, but was always removed before the onset of activity. Strips in moist air, after brief immersion in a calcium-containing solution, showed spontaneous activity in six cases out of eight that were studied. The contractions were infrequent in every case, and came at irregular intervals, although the individual contractions were very vigorous. The average duration of the series was about one and one-half hours. No decline in vigor occurred at its close. After the strip had stopped beating, moistening it with a calcium-containing solution usually called forth four or five vigorous beats close together, but had no further apparent effect. Transfer to a 0.7 per cent sodium chloride solution promptly resulted in a good sodium chloride series. In only two cases was the author able to elicit spontaneous contractions from strips which had been removed from 0.7 per cent sodium chloride to moist air before the

onset of activity. In each of these cases the total number of contractions was very small, and they appeared at long intervals.

THE REACTIONS OF STRIPS IN CANE SUGAR SOLUTION ISOTONIC  
WITH 0.7 PER CENT SODIUM CHLORIDE SOLUTION.

Fresh strips placed in sugar solution show only tone changes: There is at the outset the usual relaxation, which is followed by a gradual and continuous increase in tone; this tonic shortening may amount ultimately to a very considerable fraction of the original length of the strip. When a strip in this tonic contraction is removed to 0.7 per cent sodium chloride, a prompt fall in tone occurs, and unless the time of immersion in sugar has been excessive, spontaneous contractions will begin, sooner or later. In none of the author's experiments was the onset of activity after the application of 0.7 per cent sodium chloride markedly prompt. If a fresh strip be placed in 0.7 per cent sodium chloride till beats begin, and be then transferred to sugar solution, activity ceases immediately, and the same rise in tone occurs as when a perfectly fresh strip is placed in sugar solution. It will be remembered that strips removed to moist air, under the same conditions, give excellent series of contractions. Howell<sup>1</sup> has described the effect of sugar solution upon strips which have been exhausted in sodium chloride. Revival of vigor as the result of transfer to a sugar solution is usual, but not invariable. When revival occurs it is gradual and continuous until a maximum is reached which is somewhat smaller than the maximum in sodium chloride. Usually no rise in tone occurs. The series in sugar, which lasts an hour on the average, terminates ordinarily without diminution of vigor, but the last few beats are at longer intervals than the others. In a few of the author's experiments the vigor declined gradually to zero. When a strip that has come to rest after a series in sugar solution, is placed again in 0.7 per cent sodium chloride, it usually gives a series of contractions like that ordinarily seen in sodium chloride, save for the exceedingly rapid decline in vigor which occurs. In the author's experiments, the longest series given by a strip in sodium chloride after exhaustion in sugar was twenty minutes. If the strip be returned to the sugar solution after this second sodium chloride exhaustion, a second good recovery and series may ensue. This may, in turn, be followed by a short series

<sup>1</sup> HOWELL: *Loc. cit.*, p. 185.

in sodium chloride. The author has not succeeded in carrying the alternation of sodium chloride and sugar farther than this with good results. The effect of solutions containing both sodium and calcium chlorides, or of Ringer's solution, upon strips exhausted in sugar, is, as Howell<sup>1</sup> has pointed out, extremely beneficial. Excellent series of long duration may be obtained by the use of these solutions; in the author's experiments the duration of series under these conditions ranged from six to forty hours; the average was about eighteen hours. If a strip which has been exhausted in sugar be transferred to a moist chamber, it frequently revives, and gives a long and excellent series of contractions. These series showed, in the author's experiments, about the same range of duration, and the same average duration as was noted above for strips in solutions containing both sodium and calcium chlorides. This result was obtained repeatedly, and is interesting inasmuch as a strip after exhaustion in sugar has been assumed to be wanting in all diffusible ions. Moistening the strip with a sugar solution containing calcium, at the moment of placing it in the moist chamber, was without immediate effect in two instances, save for a slight rise in tone. The beats, which began after a short latent period, were vigorous, but not demonstrably more so than in cases where no calcium had been applied. In neither of these instances was the length of the series widely different from the average. In one case, however, a strip that had been exhausted successively in sodium chloride and sugar, and had then been beating feebly in a moist chamber for two and one-half hours, was moistened with a sodium-calcium solution, containing 0.05 per cent calcium chloride. The force of contraction increased promptly and markedly as a result of this application, and the strip remained active for twenty-five hours after the application of the reviving solution.

After a strip has been exhausted successively in 0.7 per cent sodium chloride, isotonic sugar solution, and moist air, and has given a good series of contractions in each, it may then be revived by being placed in a solution of sodium and calcium chlorides, and may give an excellent and vigorous series in this solution. The figures for two such experiments are as follows:

*Experiment of Dec. 17, 1903.* — A strip was exhausted in 0.7 per cent sodium chloride by 1.5 hours' immersion. It was then placed in isotonic sugar solution, in which it made a good recovery and gave a typical series of

<sup>1</sup> HOWELL: *Loc. cit.*, p. 190.

the usual length. At the end of 1.5 hours after being put into the sugar solution, it had become perfectly quiescent. It was then transferred to a moist chamber, where it recovered a second time, and remained active for more than seventeen hours. Eighteen hours after being placed in the moist chamber the strip had again come to rest. It was then put into a solution of sodium and calcium chlorides. Vigorous beats commenced at once, and the strip remained active for twelve hours.

*Experiment of Dec. 21, 1903.* — A strip was exhausted successively in 0.7 per cent sodium chloride, isotonic sugar solution, and moist air, giving a typical series of contractions in each medium. It was in the salt solution two hours, in the sugar solution one hour, and in the moist chamber twenty-one hours. At the end of this treatment it was immersed in a sodium calcium solution, containing 0.05 per cent calcium chloride and 0.7 per cent sodium chloride, and gave a good series of beats lasting fifteen hours.

#### SUMMARY.

The following reactions seem to the author to be normal for ventricular tissue of the turtle, and in his opinion must all be taken into account in any attempt to formulate a satisfactory theory of the rhythmicity of such tissue.

1. Ventricular tissue of the turtle's heart is not spontaneously rhythmical under the conditions of normal life. This is shown by the fact that it ceases to beat when completely isolated.

2. It contains within itself all the conditions essential for rhythmic activity, provided they can be brought into play.

3. By subjecting the tissue to certain artificial conditions it can be made spontaneously rhythmical; the most important of these artificial conditions are stated in the succeeding sections.

4. A sodium salt in some proportion must be present in any solution which is to be used as a bath about a ventricular strip for the purpose of initiating spontaneous contractions in it.

5. A brief preliminary bath of a solution containing calcium in physiological proportion, followed by immersion in 0.7 per cent sodium chloride, induces spontaneous beats in a fresh strip more promptly than any other solution now known.

6. Fresh strips will begin to beat spontaneously if treated only with 0.7 per cent sodium chloride, but much more time elapses before the onset of activity in this case than in the former one.

7. Spontaneous contractions may occur in solutions containing a much smaller amount of sodium chloride than that usually consid-

ered as physiological, if the solutions are kept isotonic by the addition of some "indifferent" substance, such as cane sugar, but the latent period in this case is much prolonged over that which occurs in the other cases mentioned.

8. Long continuance in the abnormal conditions incident to complete isolation from the body, modifies the tissue so that its general sensitiveness to the influence of salts is markedly increased.

9. The series of contractions in 0.7 per cent sodium chloride is characterized by continuous diminution of vigor, resulting finally in standstill, the so-called "sodium chloride exhaustion."

10. Recovery from sodium chloride exhaustion may be secured by adding calcium chloride to the solution, by permitting free diffusion of the electrolytes present in the heart-muscle, or by preventing diffusion completely. The recovery by the second method is comparatively short-lived, while that brought about by the first and third methods is very long continued.

11. Spontaneous activity in isotonic solutions containing small proportions of sodium chloride is characterized by the extreme vigor of the initial contractions. The rate is much slower than in 0.7 per cent sodium chloride, but this is fully compensated by the much greater length of the series of beats.

12. Recovery from the exhaustion which occurs in dilute sodium chloride solutions may be obtained by the application of 0.7 per cent sodium chloride, by the application of mixed solutions containing both sodium and calcium chlorides, or by the complete stoppage of diffusion which results when the tissue is placed in a moist chamber.

13. Spontaneous activity in solutions containing both sodium and calcium chlorides is characterized by the very great length of the series of beats, and also by the fact that exhaustion in such solutions is not overcome by the methods applicable to other forms of exhaustion.

14. Spontaneous activity in Ringer's solution is characterized by the great length of series it induces, and by the irregular rhythm exhibited by strips immersed in it. Cessation of activity in this solution appears more like inhibition than like true exhaustion.

15. Recovery from standstill in Ringer's solution may be obtained by the application of pure sodium chloride, or of mixtures of sodium and calcium chlorides. The series of beats after recovery with the first-named solution shows the usual characteristic of a more or less

rapid decline to zero, while that with the second solution is very long and well sustained.

16. Spontaneous activity in moist air occurs only after it has been induced by treatment with one of the effective solutions described above. The nature of the series of contractions given in the moist chamber depends upon the previous treatment which the tissue has had. The most favorable preliminary treatment appears to be exhaustion in 0.7 per cent sodium chloride, or successive exhaustion in this medium and in sugar solution isotonic with it. The momentary application of calcium to a strip in moist air affects favorably both the vigor of contraction and the rhythm of the series. The later stages of a moist chamber series are characterized by irregularity of rhythm like that shown by strips in Ringer's solution. If the strip is removed to moist air at the very beginning of spontaneous activity, the resulting series is characterized by gradually declining vigor from the outset, as well as by the diminishing frequency of contraction which is usual in moist air. The series under such conditions is much shorter than when the moist chamber is applied after exhaustion in sodium chloride.

17. Recovery from moist chamber exhaustion may be obtained in precisely the same ways as from standstill in Ringer's solution. In this case, as in that, the recovery which results from the application of a mixed solution of sodium and calcium salts is much better than that which follows the use of sodium chloride alone.

18. Spontaneous activity in sugar solution, isotonic with 0.7 per cent sodium chloride, appears to occur only after previous exhaustion in sodium chloride. It is characterized by the short duration of the series, and by the fact that standstill frequently comes on with the extent of contraction still high.

19. Recovery from standstill in sugar solution may be obtained by the application of isotonic sodium chloride, in which case the recovery is very short-lived, or by the application of a sodium-calcium solution, or by the stoppage of diffusion which results from the removal of the strip to moist air; the recovery in the two latter cases is excellent and long-continued.

#### THEORETICAL DISCUSSION.

Study of the results described above leads to the conclusion that the simple theories thus far proposed in explanation of the reactions of ventricular tissue to salts, in which all the effects observed are

attributed to mere diffusion inward or outward of one salt or another, are insufficient to account for all the phenomena, and that the ultimate explanation of these effects will prove to be more complicated. Two sets of phenomena reported in this paper seem to be peculiarly instructive when studied together. The first of these has to do with the effect of calcium on the latent period, and the second with its effect on recovery from sodium chloride exhaustion. In the first case it is seen that spontaneous contractions appear promptly in a fresh strip treated with calcium and then placed in sodium chloride, while if the calcium treatment be omitted, and the fresh strip be placed directly in the saline solution, contractions appear only after a considerable lapse of time. The second case is analogous; a strip which has lost its irritability as the result of prolonged immersion in salt solution is recovered with great promptness by the application of calcium, and exhibits an equally good recovery as the result of removal from the sodium chloride solution to moist air, but only after a long interval. These facts may be taken to indicate that the result of immersion in sodium chloride, in the first instance, and of suspension in moist air, in the second, is to bring about gradually, by processes within the tissue, conditions equivalent to those obtained immediately by the application of calcium directly to it. The tentative hypothesis which the author offers in explanation of the observations of himself and others, along the lines under consideration, is based upon the idea expressed above. The first assumption suggested would be that the liberation of energy in ventricular tissue is dependent upon the presence in it of calcium in diffusible form, but that the normal calcium-content of the tissue is for the most part in some indiffusible form. According to this assumption, one effect of stimulation upon the tissue would be to convert the calcium from its indiffusible inert form to a diffusible active form. When a strip of ventricle is isolated from its normal connections, it may be supposed that the calcium-content passes over gradually from the inert to the active form, perhaps through the action of sodium salts upon the tissue, and that activity begins when sufficient diffusible calcium has accumulated to produce its effect. Although the assumed active calcium has been designated as diffusible, satisfactory explanation of some of the observed phenomena requires the assumption that the actual diffusion of calcium is comparatively insignificant when the tissue is at rest, but marked when it is in action.

The observations recorded in the author's summary must be

examined in their relation to this hypothesis, in order to see how far it is in accordance with the facts known at this time.

**The latent period.**—The effect of the external application of a calcium salt in shortening the latent period agrees with the idea that the presence of calcium ions is essential to activity, since this treatment provides them at once, and is followed by prompt activity. The idea has been advanced that the inert calcium-content of the tissue is rendered active suddenly as the result of stimulation. It may be supposed that sodium ions applied about the strip bring about a similar transformation gradually. From this point of view the latent period in a sodium-containing solution would be looked upon as an expression of the time required for sufficient transformation of calcium to take place to induce contractions. The fact that dilution of the sodium chloride bath with sugar solution prolongs the latent period can be explained by supposing that the rate of conversion of the calcium from its inert to its active form is dependent on the amount of sodium in the external environment.

**Sodium chloride exhaustion, and recovery from it.**—Upon the assumption that diffusion of the active calcium takes place with great readiness while the tissue is contracting, the decline of vigor in sodium chloride solution follows as a natural consequence. This solution offers no opposition to free calcium diffusion whenever the condition of the tissue permits it. It may be supposed that when the tissue is active diffusion of calcium goes on more rapidly than does the process of transformation into the active form. The result would be a gradual diminution in the quantity of effective calcium, as compared with the amount of sodium present, and an accompanying decrease of vigor. Addition of calcium to the solution in which exhaustion has taken place should have a prompt and favorable effect upon the vigor of contraction, because it acts to restore at once the balance between the sodium and calcium, upon which the execution of powerful contractions seems to depend. The beneficial effect of the presence of calcium in the bathing solution would be expected to be, as it is, long continued, since in this way the favorable proportion between the sodium and calcium is maintained. When an exhausted strip is transferred to a moist chamber, outward diffusion of calcium comes to an end, but the transformation into the diffusible form may be supposed to continue. The result will be a gradual accumulation of active calcium which will be accompanied by rhythmical contractions of increasing force. When a strip, after exhaustion in sodium



chloride, is placed in sugar solution, outward diffusion of sodium is possible, as well as of the other electrolytes. If it be supposed that sodium is highly diffusible, more so than the active form of calcium, the result of the transfer to sugar would be a *relative* increase in the amount of active calcium, as compared with the sum total of the other electrolytes of the tissue, and this relative increase in the amount of effective calcium could be looked upon as the cause of the increased vigor of beat.

**Activity in sodium chloride diluted with isotonic sugar solution.**—The characteristic features of activity in dilute sodium chloride solutions, namely, great vigor and slow rate, may probably be interrelated, as Lingle<sup>1</sup> has suggested. The cause of the slow rate is not probably the same as of the long latent period, *i. e.*, the small proportion of sodium in the bathing solution, and presumably, as the result of diffusion, in the tissue also. If the possible output of energy of the strip is not affected by the diminution in the amount of sodium in it, the slower rate would naturally be compensated by greater force of contraction. It should be borne in mind in considering the effects of solutions containing sugar, that the possibility of direct influence on the part of this substance is by no means excluded, and while it is desirable, from theoretical considerations, to consider sugar solutions indifferent, the experimental results of Howell<sup>2</sup> and others indicate that they may exert a definite and specific influence upon tissues immersed in them. The most interesting of the methods for reviving strips which have come to rest in dilute sodium chloride, is the application of 0.7 per cent sodium chloride. The revival under this treatment is ordinarily very prompt in showing itself, indicating that whatever the direct influence of the sugar may be, it is quickly overcome. On the assumption that sodium influences the conversion of calcium to its effective form, the beneficial effect of the application of the strong solution of salt would be due to the fact that it brings this transformation about to an increased degree. At the same time the normal proportion of sodium would be restored in the tissue, and complete outward diffusion of the sugar would occur.

**Activity in mixed solutions of sodium and calcium chlorides.**—Howell<sup>3</sup> has described and discussed the results of immersing heart strips for a long time in sodium-calcium solutions in which the pro-

<sup>1</sup> LINGLE: This journal, 1900, iv, p. 275.

<sup>2</sup> HOWELL: This journal, 1901, vi, p. 188.

<sup>3</sup> HOWELL: This journal, 1901, vi, p. 187.

portion of calcium is somewhat greater than the physiological. He points out that the ultimate effect of this large dose of calcium is to throw the tissue into a contracted condition, which he designates as calcium rigor. He points out that after a strip has gone into this condition it cannot again be roused into action. In the present paper it was shown that in cases in which the proportion of calcium is not great enough to bring about calcium rigor, the cessation of activity is marked by the same characteristic of resistance to ordinary modes of recovery. This is what is to be expected on any theory which assumes that interaction between sodium and calcium is essential to the activity of this kind of tissue. If the proportions of sodium and calcium in the bathing solution are correct, activity might continue until the available supply of energy-liberating material is exhausted, and after that point is reached recovery can only be obtained, if at all, by renewing the supply of such material.

**Activity in Ringer's solution.**—This solution differs from the one just under consideration only in that it contains a small proportion of potassium chloride. The relative infrequency of contraction and irregularity of rhythm which characterize activity in this medium, can be most simply explained as Howell<sup>1</sup> has suggested, as due to the restraining and inhibiting influence of the potassium ions. The fact that cessation of activity is not accompanied by diminishing vigor, but only by lessened frequency of contraction, indicates strongly the influence of an inhibiting agent. That this agent is the potassium is indicated with equal clearness by the fact that the only treatment necessary for complete renewal of activity is the change from Ringer's solution to a precisely equivalent one in which no potassium salt is present.

**Activity in moist chambers.**—The fact that the freshly isolated ventricle ordinarily remains at rest in moist air can be explained as due to the inhibiting action of the potassium ions present in the tissue, or, according to the suggestion made in this paper, on the supposition that the calcium does not become effective except under the influence of sodium. It seems to me not unlikely that both these factors may be operative. All the methods by which spontaneous contractions can be initiated are explicable upon this assumption of a twofold cause of standstill, and it accords with the facts which are known as to the effects of variations of environment upon the spon-

<sup>1</sup> HOWELL: *Loc. cit.*, p. 201.

taneous activity of the strip, after it has been initiated. Two facts, first, that the later stages of series of contractions in moist chambers normally exhibit increasing irregularity of rhythm and diminishing rate, presenting, thus, more and more, the characteristic features of series in potassium-containing solutions, and, second, that cessation of activity under these conditions resembles inhibition, rather than exhaustion, seem to indicate that there may be a gradual accumulation of effective potassium ions, analogous to that which has been suggested for calcium. The methods for reviving strips which have come to rest after activity in moist air, may be interpreted as favoring such a view. As was noted in a previous paragraph, the modes of causing recovery after exhaustion in moist air are the same as those which are effective in overcoming Ringer standstill, namely, methods which provide for the removal of the excess of potassium by diffusion. A comparison of all the methods of treatment which result in a renewal of activity after standstill in moist air, shows that the failure of the strip to beat can hardly be due to lack of sodium or of calcium, or to excess of either of these substances, inasmuch as revival can be obtained by the application of mixtures of sodium and calcium, or by either one without the other. The most direct conclusion, in view of all these facts, is that excess of potassium is the cause of the cessation of activity in moist air. This conclusion involves the assumption of an accumulation of potassium, which can be most readily explained, it seems to me, by supposing the potassium to be present in the tissue in an ineffective form, analogous, perhaps, to that in which the calcium occurs normally, and that it undergoes a gradual transformation, by virtue of which it becomes effective.

**Activity in sugar solution.**—Spontaneous contractions seem to occur normally in solutions of sugar only after complete exhaustion in sodium chloride solution. Inasmuch as a bath of sugar solution allows, presumably, diffusion of all electrolytes from the tissue, and it is well established that the presence of electrolytes is one of the essentials of activity in heart-tissues, Howell's<sup>1</sup> assumption of a differential diffusion seems best adapted to explain the revival which occurs under these conditions. Since immersion in sugar solution does not cause beats under other circumstances, it must be supposed, granting that differential diffusion is the correct explanation in this case of the activity after sodium chloride exhaustion, that the condi-

<sup>1</sup> HOWELL: *Loc. cit.*, p. 198.

tion of the tissue, as regards its content in electrolytes, is not such that the differential diffusion in sugar will cause or revive contractions, save under this special circumstance of previous exhaustion in sodium chloride. According to the hypothesis in whose light the heart reactions are being considered in this paper, the end of the series in sodium chloride would be a favorable time for such a differential diffusion to be effective; the transformation of calcium to its active form is well established, the sodium content of the tissue is at a high level, and the previous bath of sodium chloride has permitted the outward diffusion of potassium. In fresh strips, or those that have been but a short time in sodium chloride solution, these favorable conditions do not occur. The cessation of activity in sugar after the short series of beats which occurs in this medium may be due to the disturbance of that relationship of the electrolytes which results in activity, through their continued outward diffusion, or it may be due to a direct effect of the sugar. The methods of restoring a strip to action after it has come to rest in sugar, particularly the transfer to moist air, which induces a most excellent recovery, seem to indicate that the standstill in sugar solution is due to disturbance of the electrolytic relationships, rather than to an injurious effect of the sugar.

**Seasonal variations in the reactions of heart tissue.**—The marked shortening of the latent period in sodium chloride exhibited by some hearts in the summer time, seems to me to be of sufficient interest to merit a brief discussion. The hypothesis under consideration supposes that irritability in heart-tissue depends upon the amount of transformation of its calcium-content from an ineffective to an effective form. It can be conceived that the ease with which this transformation may be induced differs with variations in external conditions, particularly, perhaps, with seasonal or temperature changes. If we suppose the inert calcium-content of the summer heart to be much less stable than that of the heart in cold weather, the gentle accelerating action of sodium in solution would bring about the transformation of the calcium in less time, with the resulting effect of shortening the latent period. It is difficult to imagine why increased instability of the normal calcium-content of the tissue should always be accompanied, as it seems to be, by an inhibitory influence on the part of large doses of calcium in the surrounding solution; but a more intimate knowledge of the relations of the various salts to the heart's activity will doubtless make this point clear.

## THE CHEMISTRY OF MALIGNANT GROWTHS.—FIRST COMMUNICATION.

By S. P. BEEBE.

[Contributions from the Huntington Fund for Cancer Research, Loomis Laboratory, New York.]

THE tissue of a tumor is probably typical and may be expected to have characteristics distinguishing it from the normal tissues of the body as sharply as the various normal tissues may be separated from one another. Although types of tumor cells may be found in the adult or embryonic tissues, still certain irregularities in structure may be noted; and in its activities tumor tissue forms so marked a contrast to the other body tissues that it may be expected to show chemical peculiarities as well. It has seemed advisable, therefore, to make a study of the chemical compositions of tumors.

This work was begun upon fresh material, and the present paper deals chiefly with the conditions found in certain tumors that had undergone degeneration *in situ*. The results given are those obtained from a variety of material, but the difficulty of getting suitable specimens for further work in the same line has led to the publication of the results thus far obtained.

**Tumor No. 1.**—*Carcinoma of the broad ligament.* This tumor showed marked evidence of degeneration. The whole ligament was not yet involved, so that a little apparently normal tissue was separated from the tumor, which was removed during an operation performed about six hours previously. Being very soft, it was easily ground to a fine pulp in the hashing machine. Cultures showed the pulp to be free from bacteria.

This pulp was added to three volumes of distilled water, thoroughly stirred for half an hour, a little acetic acid added, and the whole warmed to 40° C. on a water bath. The insoluble tissue-elements collected were filtered off and dried at 85° (Residue A). The filtrate was then heated to boiling for one minute to coagulate proteids. The second filtrate gave the following qualitative reactions:

1. Biuret, strong reaction.
2. Adamkiewicz, strong reaction.
3. Chlorine water for free tryptophan, strong reaction.
4. Millon, positive.
5. Reduction, Fehling and Nylander, negative.
6. Iodine for glycogen, negative.

The remainder of the filtrate was used to determine the presence of amino-acids; it was evaporated to small bulk and saturated with gaseous hydrochloric acid. No crystals appeared after six days in the refrigerator. After removal of the acid in the usual way, and further concentration, leucin and tyrosin crystallized out. The tyrosin was identified by its solubilities, crystalline form, and color-reactions; and the copper salt of the leucin was made, and its presence thus confirmed.

On still further concentrating the filtrate, large quantities of a substance having the crystal form and sweet taste of glycoll separated out. The identification of this substance as glycoll was completed by,

First, its content of nitrogen,

0.2480 gm. of the substance gave 0.0452 gm. of nitrogen, = 18.24 per cent.  
Calculated for glycoll, 18.66 per cent.

Second, on benzoylating, it gave hippuric acid, identified by its crystal form, solubilities, and melting point, 181°.

**Residue A.**—The dried residue contained 12 per cent nitrogen, a trace of phosphorus, but no sulphur. A considerable quantity of ether-soluble material was obtained from it.

4 gms. of the dried tissue yielded 0.304 gm. ether-soluble material, = 7.6 per cent.

4 gms. dried tissue, digested for 48 hours at 38°, in 0.2 per cent hydrochloric acid and pepsin, yielded 0.298 gm. ether-soluble material, = 7.45 per cent.

The ether-soluble material was saponified with sodium alcoholate, evaporated to dryness; the residue dissolved in water and shaken out with ether. The ether extract, after evaporation to dryness and washing with a little cold alcohol, was taken up in chloroform. This solution gave the color-reactions of cholesterin.

The degeneration that has taken place here is evidently one of autolysis. The products obtained are those characteristic of cleavage

by the digestive enzymes, and the tissue is sterile. If the circulation had not been impaired, the soluble products would probably not have been found in such quantities. This is particularly true of glycoll, which has thus far been found free in animal tissue in only one other case.<sup>1</sup> It should be noted, however, that in the present case the tissue is degenerated, and the tumor was growing on a structure, the broad ligament, which would be expected to yield collagen and consequently glycoll. In this respect the tumor resembled the tissue upon which it was growing, or which had been eroded by its growth.

The fact that all the fat could be extracted with ether before subjecting the tissue to gastric digestion, was to be expected, and is explained by the proteolysis which the tissue had undergone *in situ*. Taylor has found that the fat can be more completely extracted from the livers of frogs that have been poisoned with phosphorus than from the livers of normal animals.<sup>2</sup> It is a well-known fact that "phosphorus" livers autolyze more rapidly than normal livers, and it seems probable that Taylor was dealing with a tissue that was in much the same condition as the tumor just described. I am not able to see in these results any good reason for believing that fatty degeneration involves a kind of proteolysis.

**Tumor No. 2.**—*Hypernephroma*. This tumor was in size and outline much like a normal kidney, except that it was much flatter. The thin capsule was striped off, leaving a soft, white, cheesy mass of material which was easily ground to a thin pulp. Cultures showed this pulp to be free from bacteria. After extracting the pulp for thirty minutes with three volumes of distilled water, a few drops of acetic acid were added, and the mass warmed to 40°. The insoluble tissue-shreds were filtered off and dried (Residue A).

The filtrate contained no coagulable proteid. It had a pale straw color with the characteristic opalescence of a glycogen solution. It showed the following qualitative reactions.

1. Adamkiewicz, positive.
2. Tryptophan, chlorine water, strong reaction.
3. Biuret, positive.
4. Millon, positive.
5. Iodine for glycogen, very strong.
6. Fehling, no reduction.

<sup>1</sup> CHITTENDEN: *Annalen der Chemie und Pharmacie*, 1875, clxxviii, p. 266.

<sup>2</sup> TAYLOR: *Journal of medical research*, 1903, ix, p. 59.

A portion of the filtrate was treated by the method of Hopkins and Cole<sup>1</sup> to separate the tryptophan. The mercury precipitate on decomposition yielded a substance which gave the chlorine-water reaction for tryptophan. From another portion of the filtrate the glycogen was removed by precipitating with alcohol; on concentration of the glycogen-free filtrate, considerable quantities of leucin and tyrosin crystallized out.

**Residue A.**

*Nitrogen.* — 0.3990 gm. tissue gave 0.0372 gm. nitrogen, = 9.31 per cent.

*Fat.* — 1. 10 gms. tissue gave 2.919 gms. ether-soluble material.  
2. 10 gms., digested for 24 hours at 40°, with 0.2 per cent hydrochloric acid and pepsin, gave 2.885 gms. ether-soluble material.

This fatty substance was dissolved in alcohol and saponified with sodium alcoholate. After saponification, it was evaporated to dryness on a water bath, the residue dissolved in water and shaken out with ether. The ether extract was evaporated, and the residue washed with cold alcohol containing a trace of hydrochloric acid. This residue, dissolved in chloroform, gave the color-reactions of cholesterin.

1.4750 gms. of the original fat contained 0.2680 gm. of cholesterin,  
= 18 per cent.

The fat contained a small amount of phosphorus, indicating the presence of lecithin, the content of which was not determined.

The fat constants determined for the ether-soluble material before the separation of the cholesterin are the following:

Acid number, 39—.

Saponification number, 167.

Iodine number, 70.5.

In this tumor, as in number one, abundant evidence of autolysis is found. The unusual quantities of glycogen, fat, and cholesterin occurring in a tissue with tryptophan, leucin, tyrosin, and other proteid-cleavage products suggests a peculiar metabolic activity. No coagulable proteid could be obtained from this specimen. Apparently there is a continuous fatty degeneration going on during the growth of the tumor.

<sup>1</sup> HOPKINS and COLE: Journal of physiology, 1901, xxvii, p. 418.



**Tumor No. 3.** — *Angiosarcoma from the leg.* This tumor had undergone a profound degeneration. It was treated in the same manner as the two previously described. The pulp was free from bacteria. The filtrate free from the coagulable proteid gave the following reactions:

1. Millon, positive.
2. Adamkiewicz, positive.
3. Biuret, strong reaction.
4. Tryptophan, chlorine water, negative.
5. Fehling, no reduction.
6. Iodine for glycogen, doubtful.

The glycogen reaction was so peculiar that it may be of interest to describe it briefly. In the acid filtrate iodine gave a greenish color with the formation of a precipitate after some time. When the solution was made alkaline with sodium carbonate, a blue color like that of the starch-reaction was obtained.

The proteids were removed in the usual way by the Brücke-Külz reagent. The addition of alcohol to the filtrate gave a precipitate which in appearance was much like glycogen. When washed and dried the precipitate had a light-green color. It reacted as follows:

1. In neutral solution it gave with iodine a greenish color which turned blue on adding sodium carbonate.
2. When warmed a few minutes with dilute acid, and then tested with iodine, a color was obtained similar to that given by glycogen.
3. After boiling with dilute hydrochloric acid, Fehling's solution was reduced.
4. It contained nitrogen, but gave none of the proteid reactions.

After removal of the glycogen-like body from the filtrate, and concentration of the same, leucin and tyrosin crystallized out.

In the three other tumors reported in this paper tryptophan was identified by its reaction with chlorine water. Possibly the reason it was not found in this case is the fact that degeneration had proceeded further. As far as I know, a substance having the behavior of the glycogen-like body has never been described before. Its reactions suggest a colloid carbohydrate having a larger molecule than glycogen.

**Tumor No. 4.** — *Round-celled sarcoma from the shoulder.* In distinction to the other specimens, tumor number four was a tissue that gave no macroscopic evidence of degeneration. Stained sections showed a small beginning degeneration. It was treated like

the others by hashing and extracting with water. After removal of the insoluble tissue, and coagulating the proteid, the filtrate gave the following reactions:

Positive, Millon, Adamkiewicz, biuret, tryptophan (chlorine water).

Negative, Fehling.

After precipitation with lead acetate, and the removal of the lead in the usual way, the filtrate was concentrated. A small amount of material having the crystalline appearance of leucin separated, but no tyrosin could be obtained.

In these tumors products of proteolysis were found, and the tissue was sterile in each case. The degeneration noticed must have been one of autolysis. Probably the main reason why the products are found in such abundance is the impaired circulation.

# STUDIES IN BODY-TEMPERATURE.—I. INFLUENCE OF THE INVERSION OF THE DAILY ROUTINE; THE TEMPERATURE OF NIGHT-WORKERS.

By FRANCIS GANO BENEDICT.

[From the Chemical Laboratory of Wesleyan University.]

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## I. INTRODUCTION.

THE temperature of the healthy human body normally undergoes certain changes which, when graphically expressed, take the form of a curve, the characteristics of which are a fall in temperature in the evening, followed in succession by a minimum temperature somewhere between 12 midnight and 6 A.M., a marked rise in the morning and a maximum at about 4 to 6 P.M. This rhythmical fluctuation has long been observed and numerous investigations<sup>1</sup> have been made to determine the constancy of the periodicity, the time of the maximum and minimum, the total range of temperature and the causes of the fluctuations.

An examination of the curves published by the numerous writers shows a remarkable persistency in general of the salient points as

<sup>1</sup> PEMBREY, in his article on animal heat, in SCHÄFER'S Text-book of Physiology, 1898, i, pp. 785-867, has given an excellent resumé of all of the earlier work on body-temperature.

outlined above, and consequently, it is reasonable to assume that individuality does not play a very important rôle in the nature of the fluctuation, although it is to be said that individuality must not be lost sight of in interpreting any single series of observations on a single subject. Aside from the sharp coincidence of the curves obtained by Jürgensen and Liebermeister,<sup>1</sup> perhaps those showing the most regularity are given in a previous article,<sup>2</sup> in which the 24-hour curves of three different individuals all show essentially the same general conformation.

Two factors have noticeably hindered research on normal temperature fluctuations: (1) observations during the night are difficult to obtain, and accordingly are extremely few in number; and (2) inasmuch as the whole range of temperature usually observed in health is rarely over 2° C., the usual methods of thermometry are at best ill-suited for this special investigation.

By means of a combination of circumstances which obtain in this laboratory, opportunity is offered for numerous observations of body-temperature during the night hours, in connection with the long series of metabolism experiments continually in progress with a respiration calorimeter. As these experiments are frequently of from nine to twelve days' duration, a series of continuous temperature observations of the subject of the experiment is readily obtained by one of the two night-observers. Furthermore, a recently devised electrical resistance thermometer,<sup>3</sup> reading to 0.01° C., offers unusual opportunities for obtaining normal body-temperature deep in the rectum.

## II. DESCRIPTION OF THERMOMETERS.

The thermometer, which has been described in detail elsewhere,<sup>4</sup> consists briefly of a resistance coil of pure copper or platinum wire enclosed in a pure silver tube. The ends of the coil are soldered to two flexible leads passed through a hard rubber plug fitting into the end of the silver tube. The leads and hard rubber are covered with pure rubber tubing and connection is made by soldered joints to a

<sup>1</sup> JÜRGENSEN and LIEBERMEISTER: SCHÄFER'S Physiology, 1898, i, p. 800.

<sup>2</sup> BENEDICT and SNELL: Archiv für die gesammte Physiologie, 1902, xc, pp. 33-72.

<sup>3</sup> BENEDICT and SNELL: Archiv für die gesammte Physiologie, 1901, lxxviii, p. 402.

<sup>4</sup> BENEDICT and SNELL: *Loc. cit.*

two or three metre length of heavy flexible lamp-cord whose farther ends are provided with two heavy copper wires that can be dipped into mercury cups. The whole resistance is about twenty ohms, and the variations in resistance produced by fluctuations in the temperature of the coil are rapidly and accurately measured by means of a Wheatstone bridge and D'Arsonval galvanometer of special construction. The painstaking care and mechanical skill of Mr. S. C. Dinsmore, mechanic of this laboratory, has made the use of this form of thermometer possible.

**Calibration of the thermometer.** — The thermometer is readily calibrated by immersion in a bath of water which is kept at a constant temperature by hand-regulation of an electric heating arrangement. A metastatic thermometer of the Beckmann type is so set that  $37^{\circ}\text{C}$ . is about midway on the scale, and immersed in the bath. Variations in temperature above or below this point are thus readily noted, and by observing the temperature on the thermometer and the reading of the galvanometer, the electrical resistance thermometer can be calibrated in a very few minutes.

It has been found most advantageous to observe the amplitude of the first deflection on the galvanometer instead of attempting to balance the resistance by the ordinary slide-wire bridge. Variations in temperature of the bath of  $0.01^{\circ}\text{C}$ . can easily be detected. Furthermore, as the variation in resistance of pure copper or platinum increases proportionally with the temperature, it is necessary only to calibrate the resistance thermometer at two points on the mercurial thermometer for the calibration curve, when plotted, is a straight line connecting these two points.

For securing the absolute temperature, the suggestion of Richards<sup>1</sup> that the transition point of the deca-hydrated sodic sulphate,  $32.383^{\circ}\text{C}$ ., be taken as the zero has been used with great success. A metastatic thermometer made by Fuess, and calibrated by the Physikalisch-technische Reichsanstalt was arbitrarily set, and when immersed in melting sodic sulphate, the mercury column stood at  $0.671^{\circ}$ . With corrections for setting, calibre, and projecting mercury thread,  $37^{\circ}\text{C}$ . was found to correspond to  $5.297^{\circ}$ . With this thermometer as the standard, the Beckmann thermometer mentioned above was calibrated by comparison in the water bath. It was found that  $37.0^{\circ}$  corresponded to  $3.414^{\circ}$ .

<sup>1</sup> RICHARDS: Proceedings of the American Academy of Arts and Sciences, xxxviii, pp. 431-440.

The Beckmann thermometer, which is one of the less expensive makes and without a calibration certificate, is kept at this setting for use in calibrating the resistance thermometers, and having been once adjusted, no further attention is required to obtain the true temperature of the bath used for calibration. The writer would again emphasize the great advantage of the use of the transition point of sodic sulphate as a fixed point in calibrating thermometers for observations of body-temperature.

The electrical connections are such that by the use of one mercury contact, the circuits are closed. About sixty deflections (millimetres) on the galvanometer scale corresponds to  $1^{\circ}\text{C.}$ , and readings are readily made to one-half a division.

As readings taken in this way require a constant voltage in the battery circuit, we have an attachment that permits adjustment of the voltage by variations in resistance for every reading, if necessary. As a matter of fact, with two ordinary "dry" cells, adjustments are seldom necessary oftener than once an hour, and then the variations are so slight as to affect temperature-measurements by the resistance-thermometer by less than  $0.01^{\circ}$ . Readings are usually recorded every four minutes.

**Method of use.**—In practice the subject coats the silver tube and several centimetres of the rubber-covered connecting wires with vaseline, and the thermometer is then inserted 10–15 centimetres in the rectum. The flexible cable is brought out through the clothes, and connection is made with the bridge system by dipping the terminals of the cable in mercury cups. The flexibility of the whole thermometer allows almost any adjustment, and it is the universal experience of all subjects that after five minutes they are unaware of the presence of the thermometer in the rectum. With such a thermometer the subject can sleep normally, sit in any position, walk about the room (returning to the observer's table at the times when observations are to be made), or even ride a stationary bicycle or ergometer, all with no discomfort.

Of especial importance is perhaps the insurance of normal sleep, for obviously, with the ordinary clinical thermometer, rectal temperatures during sleep can only be taken at the expense of normal continued sleep.

It is unnecessary here to discuss the relative advantages of the mouth, axilla, groin, vagina, or rectum as localities for taking body-temperature, for it is conceded by all physiologists that for the pur-

pose of physiological experimentation, only temperatures taken deep in the body trunk (*i. e.*, in the rectum or vagina) are of unquestioned value. Doubtless, the temperature of the urine<sup>1</sup> at the moment it is voided is that of the inner body, but obvious difficulties are in the way of using this method for continued observations, and, valuable as the method is for special investigations, it is impracticable for work of this nature.

### III. INFLUENCES AFFECTING NORMAL BODY-TEMPERATURE.

Among the possible causes of the normal fluctuations of body-temperature, muscular activity, ingestion of food, sleep, inanition, light, external temperature, position of the body, may be mentioned. In spite of the numerous investigations on the effect of these influences, few definite conclusions can properly be drawn from the published data, and the whole question remains practically unsettled. Excessive muscular work undoubtedly produces the most striking and immediate effects, and conclusions regarding its effect may be adjudged as apparently definite, while the other possible causes are so variable and slow in their action that it is impossible to draw any sharp conclusions regarding their individual effects. Recognizing that the daily curve may be a composite result of many influences, the total effect can perhaps be studied in no better way than by inverting the daily routine of life, for in such inversion practically all of the possible causes mentioned above would be disturbed as regards the time of their action. Hours of sleep, work, light, digestion, etc., would be markedly changed, and a temperature-curve taken under such conditions would be expected to throw much light on the causes of the daily rhythm.

Observations on the influence of the inversion of the daily routine are limited to those reported by Debczynski,<sup>2</sup> Jaeger,<sup>3</sup> U. Mosso,<sup>4</sup> Buchser,<sup>5</sup> and a series made in this laboratory.<sup>6</sup>

While the original report of Debczynski's results is not accessible

<sup>1</sup> STEPHEN HALES: Statistical essays, London (2d ed.), 1731, i, p. 59.

<sup>2</sup> DEBCZYNSKI: Abstract in Jahresbericht der gesammten Medicin, 1875, x, p. 248.

<sup>3</sup> JAEGER: Deutsches Archiv für klinische Medicin, 1881, xxix, p. 516.

<sup>4</sup> U. MOSSO: Archives italiennes de biologie, 1887, viii, p. 177.

<sup>5</sup> BUCHSER: Quoted from CARTER: Journal of nervous and mental diseases, 1890, xvii, p. 785.

<sup>6</sup> BENEDICT and SNELL: Archiv für die gesammte Physiologie, 1902, xc, p. 59.

to the writer, it is inferred from abstracts and other references to his work that his results show that continuous work carried on through the night reversed the temperature-fluctuations, causing the maximum ( $37.8^{\circ}$ ) to appear in the morning and the minimum ( $35.3^{\circ}$ ) in the evening. He furthermore observed that night-watching without muscular work had a similar but less noticeable effect, causing the maximum temperature ( $37.7^{\circ}$ ) to be observed in the morning and the minimum ( $37.5^{\circ}$ ) in the evening.

Buchser,<sup>1</sup> an engineer accustomed to night-work and sleep during the day, found that his body-temperature averaged  $37.25^{\circ}$  in the morning and  $36.8$  in the evening.

The earliest extended investigation into the effect of night-work was that of Jaeger<sup>2</sup> on five young men (military bakers) whose life history was about as follows :

From their fourteenth to their twentieth or twenty-first year, they had been engaged in their trade in civil capacity. Their work began about 12-2 o'clock at night and continued to 10-12 A. M. in the day. They then rested till 6 P. M., after which followed one hour of light work. They slept after the work till midnight. After one year of military drill, they entered the military bakery in September, 1879. The observations were made over a year later (November, 1880). The daily routine of the military bakers was as follows: They worked from 3 A. M. to 4 P. M. in a heated room ( $31^{\circ}$ - $44^{\circ}$  C.), with occasional quarter-hour rests, during which the meals were taken. They had no work from 4-7 P. M. From 7-8 P. M. they had light work (preparing dough), and from 8 P. M. to 3 A. M. they slept.

From the results of these observations, Jaeger concluded that night-work could cause an inversion of the temperature-curve. However, these experiments are inconclusive for three reasons.

1. The bakers slept usually from 8 P. M. to 3 A. M., and consequently such hours of rest cannot be considered as an inversion of the daily routine of life, but rather as instances of unusually early-rising.

2. While the influence of external temperature is but little understood, it is natural to question the value of a temperature-curve made up of observations a part of the time in a room where the temperature varied from  $31^{\circ}$  to  $44^{\circ}$  C. (waking hours), and the rest of the time in normal room-temperature, *i. e.*, from  $10^{\circ}$  to  $25^{\circ}$  lower, when submitted as evidence on the influence of night-work on the daily temperature-fluctuation.

<sup>1</sup> BUCHSER : *Loc. cit.*

<sup>2</sup> JAEGER : *Loc. cit.*



3. An actual comparison of temperature-curves obtained on the five military bakers with eleven young soldiers in bed (observations also made by Jaeger) shows the characteristic features of the normal temperature, *i. e.*, evening fall, minimum during the night and morning rise to be present in both curves, though with the bakers the maximum occurs at 10 A. M.

In 1885, U. Mosso<sup>1</sup> made a series of observations on himself to study the effects of night-work and sleep during the day. By observing the rectal temperature for several days, under his usual routine of life, he obtained his normal curve. During this period he slept from 11 P. M. to 6 A. M. Two meals were taken, one at 11 A. M., and the heartier meal was taken at 6 P. M.

During the second period he worked at night and slept during the day from 11 A. M. to 6 P. M. The meals were taken at 11 P. M. and 6 A. M. During practically the whole experiment, he remained in a room at the temperature of from 12° to 17°, and when awake, he spent the greater part of the time seated at a table reading or writing. This routine was continued for four days. In spite of the inversion of the daily routine, he found that the morning rise still took place at about the same time, and that the normal curve was not inverted. However, sleep during the day caused a marked fall and getting up in the evening a noticeable rise in temperature. This rise was, in at least three of the four days of inverted routine, followed shortly by a marked fall comparable to the "evening fall."

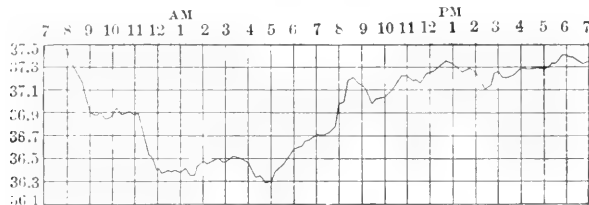
In this, the most systematic of the earlier researches, we find no evidence to imply that the inversion of the daily routine would produce an inversion of the temperature-curve.

#### IV. EXPERIMENTS ON THE INFLUENCE OF THE INVERSION OF THE DAILY ROUTINE.

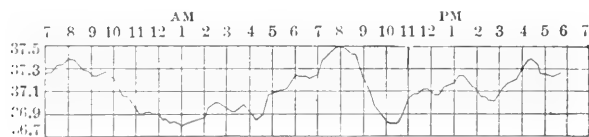
Experiments on the effect of the inversion of the daily routine may be made in two ways. A subject, usually working during the day and sleeping during the night, may be made to do the work at night and sleep during the day for a series of consecutive days, and the effect on the regular temperature-curve observed. Such an experiment was made by Mosso. Or a series of observations may be made on persons long accustomed to night-work.

<sup>1</sup> U. Mosso: *Loc. cit.*

**Experiment of 1901, with G. W. H.** — A ten-day experiment on the first plan was made in this laboratory in January, 1901, on G. W. H. who served as night-observer in connection with the metabolism experiments with the respiration-calorimeter. Several days before the metabolism experiment began, a twenty-four-hour "normal" curve (Curve I) was obtained, in which the subject slept during the night and worked during the day as usual. The form of thermometer used in these experiments allowed the night-observer to record his own rectal temperature with the same facility as he did that of the subject of the regular metabolism experiment. Consequently, on ten consecutive nights of night-work, the curves were obtained by the subject himself from 6.30 P. M. to 7.30 A. M. At 8.40 A. M. on the last day, the subject went to sleep in the calorimeter-chamber (which was vacated by the subject of the metabolism experiment at 7.00 A. M.), and the temperature-observations were continued by another observer until 6.00 P. M., thus giving a continuous twenty-four-hour record of the rectal temperature after ten nights of night-work. The individual curves for the ten successive nights are published elsewhere,<sup>1</sup> but in this connection, the two following curves are given:



CURVE I. — Fluctuations in body-temperature of G. W. H. during "normal" day.



CURVE II. — Fluctuations in body-temperature of G. W. H. on the tenth day of inversion of the daily routine.

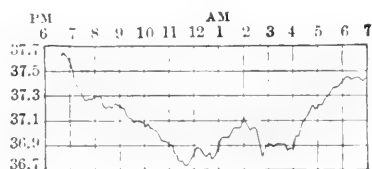
The temperature-fluctuations during the nine days preceding that of Curve II were not observed, as the subject slept outside of the laboratory on all save the last day.

<sup>1</sup> BENEDICT and SNELL: *Archiv für die gesammte Physiologie*, 1902, xc, pp. 62-67.

While Curve II cannot in any way be said to be an inversion of Curve I, it is obvious that the normal curve has undergone profound alterations as a result of the change in routine of daily life.

Obviously numerous objections could legitimately be raised to any general discussion of the subject after a single experiment of this nature, and furthermore, it must be noted that in at least one important particular the life of the subject during the days of this experiment was not a normal inversion, *i. e.*, the hours of sleep were not coincident with a twelve-hour difference from the ordinary period of rest, since, in general, the sleeping period began at 8.30 in the morning, instead of at 10.00 or 11.00 A. M., and what is perhaps more important, the hours of sleep were much shorter than the usual eight hours taken by the subject normally. In the whole period of the experiment, the sleep was seldom of six hours' duration, and more frequently four or even less. On the day in which Curve II was obtained, the subject slept soundly in the darkened respiration-chamber with, however, normal ventilation and temperature-conditions, for about six hours.

**Experiment of 1902, with G. W. H.** — During the month of April, 1902, opportunity was had to repeat a portion of the earlier experiment with G. W. H., in that a metabolism experiment with the respiration calorimeter demanded night-work for twelve consecutive nights. On the twelfth night of this work, a curve was obtained from G. W. H. that is given herewith. It was impossible to make the curve complete by continuing the observations during the sleep of the day, and consequently this omission detracts considerably from the value of the observations.



CURVE III. — Fluctuations in body-temperature of G. W. H. during the twelfth night of inverted routine of life.

The rapid fall in temperature from 6.40 P. M. to 7.30 P. M. is followed by a period of more nearly stationary temperature, a gradual regular fall completing the characteristic "evening fall" beginning at 8.50 P. M. and continuing without noticeable break until the minimum for the night is reached at 11.35 P. M. A two and one-half hour period of slowly rising temperature is followed, at 2 A. M., with a sharp fall, and after an hour of nearly stationary temperature, the regular morning rise commences at 4 A. M. A marked rise during two hours is followed from 6 to 7 A. M. by a period of nearly stationary temperature.

The whole course of the curve is strikingly similar to that of the first twelve hours of Curve I.

The fluctuations in temperature range from  $36.74^{\circ}$  to  $37.65^{\circ}$  or  $0.91^{\circ}$ , the average temperature for the period of observation being  $37.12^{\circ}$ . The maximum temperature was observed almost immediately after the experiment began, *i. e.*, 6.40 P. M., and is doubtless to be accounted for by the immediately preceding period of muscular activity on the part of the subject, resulting from the hurried walk from the dinner-table to the laboratory. The minimum for the night,  $36.74^{\circ}$ , was observed at 11.35 P. M.

With this subject the daily routine of life during the experiment was approximately as follows: on entering the laboratory in the evening, the thermometer was inserted, and the subject immediately began the duties of the night-observer. These consisted of making written records every four minutes of numerous readings of mercurial thermometers, deflections of a galvanometer, and of the muscular activity of the subject in the respiration-calorimeter in addition to a continuous alteration of resistances in electrical circuits and flow of water through pipes used to heat and cool, respectively, various sections of the calorimeter. The work, while not "muscular" in any sense, demands continued attention of the observer and precludes any dozing. For the greater portion of the time the observer remains seated in a revolving-chair, though occasionally he is required to walk to other parts of the room in connection with minor duties. About 1 A. M., a lunch is eaten. After leaving the laboratory in the morning, the subject eats breakfast and immediately goes to bed. The sleep is abnormally short and not undisturbed, for, in general, no regular sleep is obtained after 1 P. M. The time until the evening meal is taken, is occupied in reading or writing with an occasional short walk. The meal at 6 P. M. is followed by a short, brisk walk to the laboratory, where the night duties are again entered upon.

The subject when not engaged as assistant in calorimeter observations lives the life of the average college student. He is 179 centimetres high, weighs 73 kilos, without clothing, and at the time of this experiment was twenty years old. He has always been in excellent health. His previous experience as a night-worker was as follows: He worked in December, 1900, six nights; in January, 1901, ten nights; in February, 1901, ten nights; in May, 1901, ten nights; and in March, 1902, seven nights. Aside from these periods of

night-work, each of which consisted of consecutive nights, he acted as night-observer in a number of shorter periods of one or two nights: May, 1901, one night; March, 1902, two periods of two nights each; and April, 1902, one night.

#### V. GENERAL DISCUSSION OF RESULTS OF EXPERIMENTS WITH G. W. H.

It is obvious that, at least, with this subject, the influence of the inversion of the daily routine on the body temperature-curve is noticeable only during the day, for while the evening fall, the early morning minimum, and the morning rise persist, the period of sleep during the day causes a marked fall of temperature, followed by a rise on awakening.

The effect of ten consecutive nights of night-work has not therefore succeeded in producing any marked disturbance of the curve between 6 P. M. and 8 A. M. By repeating the experiment and obtaining a curve after twelve consecutive nights of night-work, no greater tendency to influence the normal course of the curve, even after the somewhat longer period of work by night is apparent.

While the data of these two curves may warrant our regarding the temperature-curve between 7 P. M. and 8 A. M. in a general way as fixed and independent of sleep or work (excepting severe muscular work), the curve during the remainder of the twenty-four hours, *i. e.*, 8 A. M. to 7 P. M., undergoes marked alterations when sleep is taken in this period. The rapid fall in temperature noticeable after the subject has gone to bed at 8.40 A. M., is the counterpart of that observed under similar conditions in Curve I, when the subject went to bed at 11 P. M.

The fall in the former case is indeed even greater than in the latter, though the observed temperature at the end of the fall is still a few hundredths of a degree above the lowest observed during the twenty-four hours, *i. e.*,  $36.78^{\circ}$  at 12.55 A. M. The maximum for the day is in the morning at 8 A. M., rather than the late afternoon, though the rise in temperature after the day's sleep continues till, at 4.24 P. M., it is within  $0.12^{\circ}$  of the morning maximum. While the average temperature of the night period in Curve II is  $37.04^{\circ}$  and that of Curve III is  $37.15^{\circ}$ , somewhat higher, neither of these temperatures can be considered as approximating the febrile condition

(37.80°) obtained by Mosso after four days of the inversion of the daily routine.

Thus it is difficult to designate as abnormal any of the conditions of life of this subject other than the short hours of sleep. Moreover, the uniformity of the two curves taken over a year apart, the one after ten days' inversion of the daily routine, and the other after twelve days of night-work and sleep during the day, leads us to believe that these curves are not abnormal for the given conditions of sleep and work. When the conformation of these curves is compared with that obtained when the subject is working during the day and sleeping during the night, we find a striking similarity that is not easy to explain, for the evening fall, minimum during the night, and morning rise persist in all cases, whether the subject goes to bed at 11 P. M., and sleeps the usual number of hours till 7 A. M., or whether he busies himself as night-observer in a metabolism experiment with the respiration calorimeter, and sleeps during the forenoon.

#### VI. EXPERIMENTS WITH NIGHT-WORKERS.

If (as is seen above) the general form of the night-curve is so firmly fixed that it is not materially altered even after an inversion of the daily routine for ten and twelve days, respectively, it would seem obvious that experiments of this nature are not best adapted for a study of this problem, and that we should resort to experiments on subjects who have been accustomed to a complete inversion of the daily routine for periods not of days or weeks but of years. Furthermore, such experiments, in order to have their greatest value, should be made on subjects whose daily routine of life is no more frequently "inverted" by night-sleep and day-work than is that of the ordinary individual by day-sleep and night-work. With this in mind, it is easily seen that but comparatively few individuals can be found whose habits of life conform to such conditions. Practically all night-workers have either days or "nights off" frequently — generally once a week — in which the hours of sleep and rest are very materially different from those of the other six days of the week. In seeking subjects for this investigation, therefore, it was necessary to find one who approximated as closely as possible the ordinary routine of the average individual save that the whole routine differed by twelve hours.

Such individuals may be found principally among night-watchmen,

though occasionally such regularity of life may be found among nurses or attendants in hospitals.

**Experiment of 1903, with A. S.** — Owing to the kindness of Mr. F. Perry Hubbard of the Rogers and Hubbard Manufacturing Company of Middletown, Connecticut, arrangements were completed so that the regular night-watchman of the factory of this company was available for temperature-observations on October 24th and 25th.

The subject, A. S., was a German, forty-four years old, weight 65 kilos, height 153 centimetres. He was apparently in excellent health, and stated that the last medical attendance was for a boil in the left axilla five years before. His experience as night-watchman covers a period of eight years, of which the last five have been of uninterrupted service in the present position. The service demanded of him requires his presence at the factory every night throughout the year. Three nights each year, two of which are consecutive, the subject procures a substitute. Other than during this brief vacation, the routine of each night is followed as outlined beyond.

His duties as night-watchman require him to be at the factory at 5.45 P. M. Every hour a tour of all the buildings is taken, and the usual watchman's time-clock is a check on the faithfulness of the performance of this duty. Each tour occupies exactly twenty-five minutes, and it is estimated that during the course of the night a distance of twelve miles, some of which is going up and down stairs, is travelled. Between tours the subject has minor duties that occupy, each night, a total of about one hour's time. Once a week the boilers are cleaned, requiring one hour's time.

When not occupied in inspecting the factory or other set duties, the subject reads in the office, or, if the weather is good, he goes outdoors and smokes. The subject spends considerable time out of doors in this way, as he finds it easier to keep awake when so doing.

Rarely he falls asleep, and if a tour is missed, it is almost always that at 2 A. M. Furthermore, if this happens one night, especial vigilance is necessary to prevent its occurrence the next night. The subject does not make it a practice to take naps between tours, finding it difficult to rely on waking at the proper moment.

The subject gets home at 6.40 A. M., and has a breakfast usually of two eggs, coffee, and bread at 7.15. Until 11.45 he is occupied with general work about the house, having a small poultry business. After a light meal of cheese, bread, and a bottle of beer at 11.45 A. M., the subject goes to bed at 12. The sleep is not as sound or undis-

turbed as when taken during the night. The subject stated that the sleep obtained in the laboratory during the two experiments reported here was deeper than he had experienced during the day in his recollection. After being awakened at 5 P. M., a warm meal is taken and the factory reached at 5.45 P. M. Occasionally a slice or two of bread with a piece of cheese is eaten during the night.

After a three-quarter mile ride on the bicycle from the house to the laboratory on October 24th, the subject entered the laboratory, inserted the rectal thermometer, and observations of body-temperature began at 5.42 P. M. With almost no omissions, the temperature was recorded every four minutes until 11.30 P. M., October 25th. The temperature of the laboratory was kept practically constant at 20° C. during the whole experiment. To throw light on the possible effect of body-position and muscular activity, a careful record was kept of the occupation of the subject all through the experiment. A summarized statement of such movements is as follows:

Sitting from 5.42 P. M.—8.02; standing and walking, 8.02–8.38; sitting, 8.38–8.54; standing and walking, 8.58–10.22; sitting, 10.26–11.10; standing and walking, 11.10 P. M.—12.14 A. M.; sitting, 12.18–12.26; standing and walking, 12.30–1.58; sitting, 1.58–2.26; walking, 2.26–3.34; sitting, 3.38–4.06; standing, 4.10–5.34; sitting, 5.34–6.02; standing, 6.02–7.46; eating breakfast, 7.50–8.02; walking, 8.06–9.02; sitting, 9.02–10.22; goes to bed, 10.30; sleeping, 10.30 A. M.—5.58 P. M.; walking, 6.02–6.18; eating, 6.18–6.34; standing and walking, 6.38–11.00; sitting, 11.00–11.34, when the experiment ended.

At 3 A. M. and again at 5 A. M., the subject ate one roll. The breakfast at 7.50 A. M. consisted of one-half mutton chop, two large cups black coffee, and two slices of bread. Supper at 6.18 P. M., consisted of steak, fried potatoes, coffee, and bread, and was the heartiest meal of the day.

Although the subject said that he had slept unusually well, it was observed that at no time was the body in perfect repose for more than twenty consecutive minutes, although the movements were not such as characterize the sleep as distinctly abnormal. Indeed, the rate of respiration, deep breathing, and low pulse-rate, as well as the statement of the subject himself, would indicate deep sleep. The incidental observations during the sleeping period are here given.

11.52 A. M., 15 $\frac{1}{2}$  respirations per minutes; 12.47 P. M., respiration-rate, 12; 3.03, respiration-rate, 12; 3.26, respiration-rate, 12; 3.30,

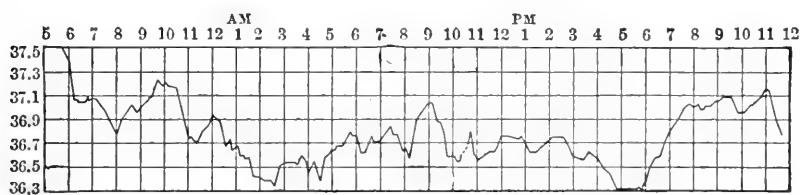


moved and asked observer the hour; 3.53, respiration-rate,  $11\frac{1}{2}$ ; 4.15, sleeping very heavily; 5.46, awake and talking. Pulse, 58; 5.58, out of bed.

The exercise indicated by the word "walking" consisted of standing and moving about in one end of the laboratory room, at no time going more than 8-10 feet from the observer's table. Walking is meant to signify more that the subject was not standing perfectly still, than that he was pacing about the room. For the most part it consisted of a shifting of the weight from one leg to the other, accompanied by an occasional step or two, and must not be interpreted as continuous motion of forward progression.

When sitting, the subject read for the most part, while, when standing, he was frequently in more or less active conversation with the observers. While sitting, and just before the experiment closed at 11.24 P. M., the pulse was 74.

The temperature-observations, which were taken every four minutes, are given in Curve IV, although the scale of the curve is not such as to render the minor fluctuations significant.



CURVE IV. — Fluctuations in body-temperature of A. S. (October, 1903).

The rapid fall observed immediately after the experiment began may be attributed to the cessation of the rather active muscular exercise involved in the short bicycle ride to the laboratory. During this period the subject was sitting in a chair near the observer's table. The rise beginning at 8 P. M. is coincident with the upright position assumed by the subject, which was continued till 8.38, when, on being seated, a slight fall in temperature is noted. Standing at 8.54 is coincident with a resumption of the upward tendency. The sharp fall at 10.22, continuing until 11.10, took place while the subject was seated. The minimum temperature for the night period was reached at 2.26 A. M., after the subject had been seated about one-half an hour. Two hours later, the temperature fell nearly to the same point, though during this period, the subject was standing or

walking. The general morning rise is broken by two periods of falling temperature from 5.45-6.10 A. M. and from 7.26-8.06 A. M., during both of which periods the subject was seated. The sharp rise of  $0.48^{\circ}$  from 8.06-9.02 A. M. was coincident with a period in which the subject was standing or walking.

The maximum of the forenoon is reached at 9.02, when the subject again sat at the desk and read. The temperature fell during the next hour  $0.49^{\circ}$ . A slight rise accompanied the preparations for going to bed, and one of the most striking features of this curve is the absence of the noticeable fall in temperature on retiring. The slight fall here observed, amounting to about  $0.22^{\circ}$ , is in no way comparable to that obtained with all other subjects in this laboratory after going to sleep. The progress of the curve during sleep is without special feature until the marked fall, beginning at 3.40 P. M. and continuing till 4.50. It is also noteworthy that from 4.50-5.45, the body-temperature, the lowest observed during the whole experiment, was constant to within  $0.02^{\circ}$ , a longer period of constancy than we have ever before observed.

On awakening the usual sharp rise is observed which continued uninterrupted till 7.55, after which follows a two-hour period of slowly rising temperature. Although the subject was standing and walking from 6.38-11.00 P. M., a fall in temperature is noted at 9.30 P. M., the upward tendency of the curve being resumed at 10.00 P. M. At 11.04 P. M. a sharp fall begins, which continues till the end of the experiment. During this last period, the subject was seated and reading.

Omitting the period of high temperature during the first few minutes of the experiment, the temperature is high at 9.48 P. M., 9.00 A. M., and again at 11.00 P. M. The lowest temperatures are observed at 2.26 A. M., and 4.50-5.45 P. M. The average temperature for the whole period (thirty hours), 5.45 P. M., October 24th, to 11.30 P. M., October 25th, is  $36.76^{\circ}$ . The average temperature for the night period, 7 P. M.-7 A. M., is  $36.76^{\circ}$ , and the average temperature for the last twenty-four hours of the experiment, 11.30 P. M.-11.30 P. M., is  $36.70^{\circ}$ . The minimum temperature is  $36.30^{\circ}$ , and the maximum  $37.50^{\circ}$ , showing a temperature-fluctuation during the whole experiment of  $1.20^{\circ}$ . If, however, the first eighteen minutes of the experiment are rejected, the maximum is  $37.30^{\circ}$ , and consequently the total temperature difference is  $1^{\circ}$ . The high temperature incidental to the work of riding the bicycle to the laboratory justifies the rejection of these preliminary readings.

While it is obviously next to impossible to produce an exact inversion of the daily routine, it is believed that the daily life of this subject is as close an approximation to a perfect inversion as can ordinarily be secured. For while many night-workers sleep in the forenoon, which corresponds to sleep during the early evening, and rise in the early afternoon, corresponding to rising at from 2-4 A. M., this subject sleeps ordinarily from 12 noon to 5 P. M., and on the day of the experiment, from 10.30 A. M. to 6.00 P. M., a custom corresponding more nearly to the hours usually spent in sleep by the average individual.

With this subject, as with G. W. H., it is to be noted that the hours of sleep are very much less than that usually taken by the average man. Usually, the subject, A. S., is in bed but five hours, and in allowing him to sleep longer than usual, it may be said that this temperature-curve is not normal for this subject. The disturbance of the temperature-curve that may result from an unusually long period of sleep is probably no greater than that of the enforced muscular inactivity required of all subjects of experiments on body-temperature. Furthermore as the object of research of this nature is not so much to obtain a curve that may be designated as "normal" for any individual, as it is to study the influence of the various factors that determine the course of the regular temperature-curve, the enforced muscular inactivity and the longer period of sleep should not be considered only as disturbing factors. As will readily be seen later, these apparent abnormalities aid materially in interpreting the cause of the various curves. It is especially significant that had the subject been waked five hours after going to sleep, the minimum temperature for the day would probably have been that observed at 2.25 A. M., and the long period of low temperature, from 4.50 P. M.-5.45 P. M., would not have been observed.

The arrangement of the meals with this subject is markedly different from that of ordinary life, for while the breakfast, in this particular case the heartiest meal of the day, is taken at 5.15 P. M., there is no regular meal till fourteen hours later, *i. e.*, 7.15 A. M., and the lightest meal of the day is taken just before retiring, at 11.45 A. M. In so far as the ingestion of food influences body-temperature, it is necessary, therefore, to bear in mind the fact of the unusual times of eating when interpreting this curve, although, as is seen from the record on p. 157, the meal hours during the experiment did not coincide with those of the usual day with this subject.

The muscular activity of the subject was much less than that to which he was accustomed, and consequently while Curve IV unquestionably does not represent the fluctuations in temperature of the body of this subject during a night of service as night-watchman, it is, in a way, comparable to those obtained on other subjects with the usual enforced quiet incidental to experiments of this nature. It should be noted in this connection, however, that owing to the considerable time during which the subject was standing or walking (see p. 158), the muscular activity was undoubtedly considerably more than that of the majority of subjects of experiments upon which continued body-temperature observations have been made. Indeed, it might appear from the marked influence of the variations in body-position, as when standing or sitting, especially as exhibited in Curve IV, a direct comparison of this curve with those upon subjects who were either lying or sitting down during the period of observation is attended with some degree of inaccuracy.

Finally it must not be forgotten that individuality may account for minor fluctuations in the course of the curve, though, as was previously stated, different individuals under like conditions of bodily activity furnish temperature-curves of remarkable similarity. Therefore, while the nature of this curve may be somewhat marked by the individuality of the subject, all previous experience would lead one to consider that under like conditions this curve would not vary widely from an average of curves from a number of individuals.

The great difficulty in securing normal healthy subjects, with habits of life exactly similar to those of this subject, precludes, at least for the present, any great elaboration of this study, but opportunity was taken to repeat the temperature-observations on the same subject four months later.

**Experiment of January 30-31, 1904, with A. S.**—No material change had been made in the subject's mode of life since the last series of observations were obtained, and there is no reason for believing that the subject was not in his normal condition. Accordingly, an experiment began at 7.30 P. M., January 30th, and continued uninterrupted till 11.30 P. M., January 31st. In order to insure as nearly as possible identical conditions of bodily activity in both experiments, an attempt was made to regulate the movements by having the subject repeat as nearly as possible each change of position that might affect muscular activity at the hour at which the change was made in the previous experiment. The programme of the experiment was

duplicated exactly from the beginning of the experiment till 6.20 P. M. on January 31st. At whatever hours the subject stood, walked, sat, or slept in the previous experiment, he was notified by the observer to assume the same body-position and maintain it until notified again. To insure comfort to the subject, the strictest adherence to the programme was not insisted on, and when sitting, no attempt was made to eliminate or equalize minor muscular movements, such as turning the pages of the newspaper or book or engaging in conversation. Likewise, when standing or walking, it was considered that the movement of walking was confined to so small an area that it would be impossible to distinguish between standing perfectly still, shifting the body-weight from one leg to the other, or moving a few paces from one point in the laboratory room to another. The time of defecation was not alike in both experiments, and some irregularity in the time of micturition was naturally to be expected. It was not deemed advisable furthermore to adhere to exactly the same diet, although as nearly as could be judged (actual weighings were not made) the total amount of food eaten at each meal did not differ materially in the two experiments. In accordance with his usual custom, the subject smoked small amounts of tobacco from time to time, and no regularity was observed in this.

During the sleeping period, careful observations were made of the movements, though, owing to the position assumed by the subject, it was difficult to obtain the rate of respiration with accuracy, and accordingly, it is not here given.

Owing to a misunderstanding, the subject was told to sit and read from 6.52 P. M. to 8.06 P. M. on January 31st, whereas he should have stood during this period. From 8.06 P. M. to the end of the experiment the previous programme was strictly followed.

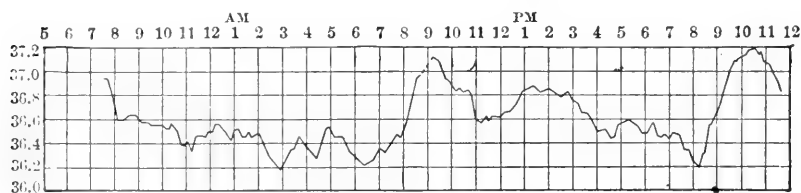
The subject entered the laboratory at 5.30 P. M., January 30th, but owing to a delay in the calibration and readjustment of the thermometer, observations did not begin till 7.30 P. M. At 11.10 P. M., and again at 2.50 A. M., a biscuit and cruller were eaten. The meal at 8.10 A. M. consisted of one cup (400 c.c.) of hot, black coffee, baked beans, one slice of bread, and one cruller. The subject fell asleep almost immediately after getting into bed at 10.32 A. M., and though the subject was no less restless than in the previous experiment, the breathing was deep, the respirations not far from 10-12 per minute, and the subject considered that he had had an unusually restful sleep.

The pulse at 6.00 P. M., just before rising, was 56.

Supper, at 6.18 P. M., consisted of one mutton chop, fried potatoes, one slice of bread, and hot, black coffee. At 8.10 P. M., just before standing, the pulse was 61.

The temperature-observations were made in general every four minutes, and they are plotted in Curve V, on the same scale as that used in Curve IV.

The rapid fall in temperature, beginning immediately after the insertion of the thermometer, continued till 8.06 P. M. During the period of approximately constant temperature, from 8.06-10.20 P. M., the subject was standing and walking. The fall in temperature at 10.20 P. M. followed the change in position of the subject, from standing to sitting. The temperature-fluctuations during the next three hours are all within  $0.20^{\circ}$ , and the first marked change is a fall from 2 to 3 A. M. The minimum for the whole experiment is observed at 2.54 A. M., though again, at 6.24 A. M. and 8.15 P. M., the temperature falls to within  $0.04^{\circ}$  and  $0.02^{\circ}$  of the absolute minimum. The sharp rise beginning at 6.24 A. M., continued till 9.18 A. M., when the subject sat and read till 10.30 A. M. On going to bed, the temperature of the subject



CURVE V. — Fluctuations in body-temperature of A. S. (January, 1904).

fell  $0.23^{\circ}$ , a fall that is very much less than that observed on the other subject (G. W. H.) after getting into bed (see Curve I at 11 P. M. and Curve II at 8.40 A. M.). After one hour of nearly stationary temperature, a rise of  $0.20^{\circ}$  takes place, followed by two hours of nearly constant temperature. From 2.30 to 4.30 P. M. a fall in temperature is noted, and the temperature,  $36.45^{\circ}$ , is the minimum during the period of sleep. On getting out of bed at 6 P. M., no such marked and rapid rise as is seen in Curve IV takes place, and, indeed, after a very slight rise, the temperature falls continuously till 8.06 P. M., when  $36.20^{\circ}$  is reached. A marked rise, amounting to  $1^{\circ}$ , and continuing for two hours, is coincident with the change in position of the subject from sitting to standing and walking. At 10.36 P. M. while the sub-

ject was still standing, the temperature began to fall, and at 11 P. M., on resuming his chair, the temperature fell steadily till 11.34, the close of the experiment.

Three maxima are observed, at 7.34 P. M. (the beginning of the experiment), at 9.06 A. M., and at 10.34 P. M., the latter observation ( $37.19^{\circ}$ ) being the absolute maximum for the whole experiment.

Three minima, all within  $0.04^{\circ}$  of each other, are observed at 2.54 A. M., 6.22 A. M., and 8.06 P. M., the first of these being the absolute minimum,  $36.18^{\circ}$ , for the whole experiment. The average temperature for the whole period (28 hours), 7.30 P. M., January 30th to 11.30 P. M., January 31st, is  $36.58^{\circ}$ . The average temperature for the night period, 7.30 P. M.-7 A. M., is  $36.50^{\circ}$ , and the average temperature for the last 24 hours of the experiment, 11.30 P. M.-11.30 P. M., is  $36.58^{\circ}$ . The minimum temperature is  $36.18^{\circ}$ , and the maximum,  $37.19^{\circ}$ , showing a temperature fluctuation during the whole experiment of  $1.01^{\circ}$ .

While from the previous experience in repeated experiments with other subjects under like conditions of daily regime,<sup>1</sup> we might expect this curve to be more nearly an exact duplicate of Curve IV than it actually appears, it is seen, at least in the essential points, to be strikingly comparable with the earlier curve, for, as is shown in the tabular statement, the times of minima and maxima, the total fluctuation and the average temperature of the last twenty-four hours, agree very closely in the two curves.

	Curve IV.	Curve V.
Minima . . . . .	2.26 A. M. 4.30 A. M. 5.50 P. M.	2.54 A. M. 6.22 A. M. 8.06 P. M.
Maxima . . . . .	9.48 P. M. 9.00 A. M. 11.00 P. M.	7.34 P. M. 9.06 A. M. 10.34 P. M.
Temperature difference for the whole experiment . . . . .	$1.01^{\circ}$	$1.00^{\circ}$
Average temperature for the last 24 hours	$36.70^{\circ}$	$36.58^{\circ}$

The similarity would probably have been even more striking had the programme of the first experiment been duplicated exactly in the second, for we would have expected the rise during the last evening

<sup>1</sup> BENEDICT and SNELL: *Archiv für die gesammte Physiologie*, 1902, xc, pp. 36-41.

to begin as soon as the subject assumed a standing position, and this change of position was erroneously delayed over an hour.

Thus, while the minor fluctuations in temperature are not always coincident, the grosser changes do not vary widely in the two curves.

#### VII. DISCUSSION OF RESULTS OF EXPERIMENTS WITH A. S.

Although definite plans are made for further research into this most perplexing problem, a few words of general discussion, though admittedly based on rather meagre experimental data, and consequently liable to subsequent revision, may not be out of place.

The remarkable course of these curves certainly cannot readily be explained as the resultant of the influences ordinarily considered as affecting body-temperature. Obviously we have here to do with some influences other than the ingestion of food, muscular activity, sleep, and body-position. Of the main characteristics of the normal curve (Curve I), the evening fall, the early morning minimum, and the morning rise, are unmistakably found in both of these curves. The evening fall, though considerably diminished in amplitude, is sufficiently well marked to be easily recognized, and the early morning minimum and morning rise are likewise clearly seen.

As with G. W. H. in Curves II and III, the general form of the night-curve remains practically intact, thus indicating a fixity of rhythm that is difficult to explain. Why the temperature of the human body reaches a minimum at 2-6 A. M., independent of whether the subject is sleeping soundly and in the recumbent position, or whether he is awake and sitting, or even standing and walking, is a problem that calls for extended research.

The important rôle played by muscular activity has led some investigators to consider that all temperature-fluctuations are incidental to muscular movement, either voluntary or involuntary. Johansson<sup>1</sup> by fasting and enforcing muscular rest succeeded in diminishing the total temperature-fluctuation in the twenty-four hours to 0.40° C., but the curve showed distinctly the presence of the evening fall and the morning rise. Hörmann,<sup>2</sup> from a continuous series of observations of vaginal temperature of an insane woman who remained in bed three days without food, concludes that muscular

<sup>1</sup> JOHANSSON: *Skandinavisches Archiv für Physiologie*, 1898, viii, p. 85.

<sup>2</sup> HÖRMANN: *Zeitschrift für Biologie*, 1898, xxxvi, p. 319.



activity and digestion are the only causes of body-temperature fluctuation. It is interesting to note that in two of his three experimental days the evening fall and morning rise are distinctly noticeable.

It is clear that the experiments with A. S. here reported furnish suggestive evidence as to the rationality of conceding that there is an influence of the time of day independent of muscular activity. This view, advanced so frequently in the older literature, has been strenuously combated, but the night periods in Curves IV and V exhibit fluctuations that are entirely opposed to the theory that muscular activity is the sole cause of temperature-fluctuation. That there is a periodicity resulting from a habit long established in succeeding generations is not unreasonable to suppose, though, until the other known factors affecting body-temperature have been more carefully studied, and their effect eliminated so far as possible, it is necessary to bear in mind, when drawing any conclusions, that the curves with A. S. are the resultant not of one but of many influences.

One of the most suggestive features of these curves is the immediate effect of change in body-position on the course of the body-temperature. Sudden fluctuations in temperature always accompany severe muscular work, — fluctuations amounting at times to over one centigrade degree in an hour; but none of the muscular movements of A. S. can reasonably be classed under the head of muscular work, and it seems fitting to designate the movements as changes in body-position.

A comparison of the statements regarding changes in body-position, as given on pages 158 and 164, with the temperature-curve at the corresponding hour, shows that almost invariably a change in position from standing to sitting, or *vice versa*, produced marked changes in body-temperature. A rise in temperature accompanied the change from sitting to standing, and a fall when the change was made from standing to sitting. This is most markedly shown on both curves by the sharp rise beginning at 8.00 A. M., when the subject stood up. At 9.00 A. M. the temperature stops rising, and a rapid fall accompanies the resumption of the sitting posture by the subject. Again at 8.06 P. M., on Curve V, the temperature, which had previously been actually falling, began to rise rapidly when the subject stood, and within two hours the body is one degree warmer.

Owing to the fact that the subject was used to considerable activity during the night, it was thought best not to restrain him, and thus cause him to be restless or uncomfortable during the experiment.

Consequently, the changes in body-position, while practically free from what would be termed muscular work, were numerous. That these movements influenced the temperature-curve as they did is a striking demonstration of the fact that body-temperature-curves, to be compared, should be made from observations under similar conditions of muscular activity. The rôle played by minor muscular movements in heat-production has been greatly underestimated, but data are being accumulated to show that even the smallest movements of the hands or arms are of importance in comparing experiments in which the transformations of energy are studied. Since body-temperature is the resultant of all those factors that determine heat-production (thermogenesis) on the one hand, and those that determine heat-emission (thermolysis) on the other, it is easily seen that a disturbance of any factor would cause a fluctuation in body-temperature. Viewed in this light, the complex nature of the conditions determining body-temperature render still less secure the ground for clear deductions from experiments in which the maximum number of factors has not been eliminated. Thus, in both Curves IV and V the "morning rise" is certainly accentuated by the sharp rise from 8 to 9 A. M. mentioned above. In fact, we may consider many of the features of the night-portions of the curves to be the result of changes in body-position. On the other hand, it is equally clear that the evening fall and morning rise in Curve I (G. W. H. during a normal day) were accentuated by the changes in body-position involved in going to bed at 11 P. M. and getting up at 7.30 A. M.

The changes in body-position apparently play so important a rôle in determining the course of Curves IV and V, that the few instances where such changes were not followed by corresponding changes in body-temperature offer sharp contrasts that demand attention. The fall in temperature after 10.30 A. M., when the subject went to bed, is in no sense comparable to the marked fall in temperature observed on G. W. H. in Curves I and II at 11 P. M. and 8.30 A. M., respectively. Here, obviously, with A. S. a change in position, *i. e.*, from sitting to lying in bed asleep, exercised a minimum influence on the temperature-curve; while with G. W. H., such a change in position was accompanied by a marked fall in temperature. Observations during sleep offer very little by way of explanation of the rapid fall after 3.40 P. M., or the hour of stationary temperature, 4.50-5.45 P. M., on Curve IV. This long period of constancy is unusual, for it is to be remembered that temperature-observations were recorded every four minutes.

The error in adjusting the body-movements of the second experiment to those of the first is primarily the cause of the interesting observations from 6 P. M. to 8.06 P. M. in Curve V. After getting out of bed at 6 P. M., the subject was seated practically all of the time till 8.06 P. M., when he stood. In spite of the fact that, during this period, the subject ate supper, drank 400 c.c. of hot coffee, and the minor muscular movements were unusually numerous, the temperature of the body continued to fall until the subject assumed the standing position. This change in position was accompanied by a rise so rapid that it is comparable only to those obtained when studying the effect of severe muscular work. Although in Curve I, G. W. H. was seated immediately after getting out of bed, from 7.30 A. M. to 8.30 A. M., the temperature nevertheless rose rather than fell. Here again the absence of any apparent effect of the change in body-position on the temperature-curve is conspicuous.

It is thus seen that while years of night-work have not succeeded in eliminating the tendency to an evening fall, a minimum some time during the night, and the morning rise, the whole course of the curve is markedly different from any with which we are familiar, and any study of the factors influencing the course of the curve must include a large number of experiments in which the habits of life, times of eating, and muscular activity should all be as nearly alike as possible in order to compare the results with the normal curves given by the several writers on body-temperature.

No tendency to an inversion of the temperature-curve by inverting the daily routine of life is observed in any of the experiments reported here.

The importance of minor muscular movements, especially those involved in a change in body-position, as influencing body-temperature, receives here a new significance, although the lack of uniformity in the coincidence of temperature-changes and changes in body-position renders the problem of analyzing a temperature-curve still more complex.

### **CORRECTION.**

Line 9 on page 446, Volume X, should read :

"addition of the neutral salts of weak monobasic, dibasic, and tri-basic acids."

# THE INFLUENCE OF EXTERNAL HEMORRHAGE ON CHEMICAL CHANGES IN THE ORGANISM, WITH PARTICULAR REFERENCE TO PROTEID CATABOLISM.

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<sup>1</sup> Dr. HAWK's share of the work in collaboration (during the first metabolism experiment and the first part of the second metabolism experiment) furnished the experimental data for a thesis offered by him, last June, in partial fulfilment of the requirements for the degree of Ph.D. at Columbia University (*Biochemisches Centralblatt*, 1903, i, p. 705). The remainder of the work has lately been completed by Dr. GIES, with the occasional assistance of Messrs. DAVENPORT WHITE, H. M. HAYS, and CHRISTIAN SEIFERT, to whom we are greatly indebted for the aid given us.

## I. INTRODUCTION.

SUDDEN losses of relatively large quantities of blood bring about profound changes in the organism. These changes appear to be relatively harmless when the amount of abstracted blood is not too great, or if the loss is not repeated at too frequent intervals. For a long time our knowledge of the character and extent of the metabolic modifications during acute anæmia was very slight and indefinite. During the centuries in which blood-letting was regarded as the "universal remedy," many deductions were made, respecting its influence on the body, which failed to stand the test of subsequent investigation. In recent years Hayem, Luzet, Ehrlich, Howell, Vierordt, Löwit, Antokonenko, Dawson, Willebrand, Baumann, and many others have given us much valuable information on the effects of hemorrhage upon the characters of the retained blood,—its general composition, the number and character of the corpuscles and their regeneration, percentage-content and change in quantity of hæmoglobin, relative coagulation-time, etc. At present, however, in spite of experimental observations by several investigators, we know very little of the obscure *chemical* alterations induced in healthy bodies by considerable losses of blood.

Late in 1902 there was begun, in this laboratory, a series of studies of the metabolic effects of hemorrhage.<sup>1</sup> Several of these investigations are now in progress.<sup>2</sup> We present here the results of our first study of the chemical changes induced by the removal of fairly large quantities of blood from healthy animals. Our initial results in this connection are chiefly such as refer to proteid catabolism after withdrawals of blood from dogs under the influence of an anæsthetic. Our purpose in this particular research has been to study the effects of loss of definite amounts of blood under conditions comparable to the acute anæmia following some surgical operations under general ether anæsthesia. In a subsequent publication from this laboratory

<sup>1</sup> Preliminary reports of the experiments here described have been published in the Proceedings of the Society for Experimental Biology and Medicine, American Medicine, 1903, vi, p. 734, and in the Proceedings of the American Physiological Society, This journal, 1904, x, p. xxviii.

<sup>2</sup> The results of one of them have already been given in a preliminary report. See POSNER and GIES: Proceedings of the American Physiological Society, This journal, 1904, x, p. xxxi.

we hope to present the results of a similar study of the metabolic effects of hemorrhage conducted with the aid of *local* anæsthetics.<sup>1</sup>

We were attracted at the outset to this particular phase of the problem of posthemorrhagic effects on metabolism, by the very obvious lack of agreement in the results obtained by previous investigators. Bauer, Jürgensen, and some of their colleagues, working with dogs, noted *increased* elimination of nitrogenous matter after hemorrhage. On the other hand Skvortsov and others, lately Ascoli and Draghi, observed *decreased* excretion of nitrogenous products from dogs under similar circumstances. Under various anæmic conditions in man, also, as in chronic and pernicious anæmia, chlorosis, after internal hemorrhage, and the like, Pettenkofer and Voit, von Noorden, Ketscher, Lipmann-Wulf, Moraczewski, Kolisch, and Ascoli and Draghi are among those who have obtained metabolic results showing decided disagreement qualitatively and quantitatively.

That the general lack of harmony in the results of previous investigations is due largely to the fact that the feeding conditions were very irregular and unsatisfactory in nearly all the experiments, is evident from a review of the papers by these authors. Then, too, the periods of observation were far too short in most cases. Even in the researches on dogs little attention seems to have been given to the careful maintenance of various conditions which are necessary for the proper conduct of metabolism experiments. In our own work we have endeavored to carefully control all such important matters of detail.

## II. DESCRIPTION OF THE EXPERIMENTS.

We had two types of experiments. Three long experiments were carried out in which we studied, in a comparative way, various phases of metabolism in dogs, before and after hemorrhage. We also determined the immediate effect of loss of blood on flow of urine by directly collecting the fluid excreted through the ureters of several dogs under anæsthesia.

<sup>1</sup> It would be impossible, we think, to improve such a study, in an animal like a dog, by conducting the hemorrhage without an anæsthetic. There would be not only the objection of inhumanity in the proceeding, but under such conditions, also, secondary factors would be present, — pain and the resultant psychical effects. The latter would doubtless be more difficult to check than the effects of anæsthesia.

**Conduct of the metabolism experiments.**—Our metabolism experiments were conducted by the general methods recently described in a paper from this laboratory.<sup>1</sup>

**Animals and environment.**—All of these experiments were performed on healthy, full-grown dogs. The dog under observation was confined in a cage<sup>2</sup> well-arranged for the comfort of the animal and adapted to the collection and separation of urine, fæces, and cast-off hair. The structure of the cage at the top permitted free circulation of air. The experiments were conducted in a well-lighted and thoroughly ventilated room. The temperature of the room was fairly uniform throughout each experiment.

Only such animals as were particularly well suited to our experiments were finally selected after trial periods. In each case we were fortunate in possessing a strong dog; one not only of a lively and playful disposition to begin with, but one, also, that seemed to be contented with his environment and treatment.

**Food.**—The food consisted of a mixed diet containing hashed lean beef, lard, bone ash,<sup>3</sup> and water. The raw meat was preserved in a frozen condition.<sup>4</sup> The commercial cracker meal was kept dry in tightly closed jars. Lard was obtained in small quantities, and was always fresh at the time of use. The bone ash consisted of the thoroughly incinerated, carbon-free, commercial product. Ordinary tap water was taken.

In each of our experiments the dog was given his daily portion of food at 5 P. M. The solids and the water were intimately mixed, and the soupy mass thus formed was eaten with very evident relish.<sup>5</sup>

**Periods, weights.**—In the records of our experiments each day ended at 5 P. M., at which time, just before the food was given, the weight of the dog was taken. The figures for weight in our tables represent, therefore, the weight of the animal at the *end* of the day of record. The daily analytic data, also, are recorded for the twenty-

<sup>1</sup> MEAD and GIES: This journal, 1901, v, p. 106. Also GIES and collaborators: Biochemical researches, 1903, i, Reprint No. 21.

<sup>2</sup> GIES: Proceedings of the American Physiological Society, This journal, 1904, x, p. xxii.

<sup>3</sup> *Ibid.*

<sup>4</sup> GIES: This journal, 1901, v, p. 235. Also GIES and collaborators: Biochemical researches, 1903, i, Reprint No. 1.

<sup>5</sup> The food was so ardently desired that it was necessary to use force to prevent the animal from spilling the contents of the dish when it was brought into the cage. The only exceptions, due to hemorrhage, are noted on pages 185, 186.



four hours ending at 5 P. M. The new periods of record, after preliminary establishment of nitrogenous equilibrium, always began with the day on which the dog was subjected to the new condition.

*Anæsthesia, surgical operation, and blood-letting.*—The withdrawal of blood was usually begun shortly after 9 A. M., and was conducted under very light ether anæsthesia, after preliminary use of a small amount of chloroform with the ether. At this time of the day the dog's stomach was practically empty. There was never any vomiting as a result of the administration of the anæsthetic. Recovery from anæsthesia was rapid and satisfactory in all cases, and by 5 P. M. the dog manifested his usual appetite.<sup>1</sup> The anæsthetics we used were chemically pure. Each anæsthesia throughout the experiment was induced with samples from the same supply of the anæsthetic.

Before this investigation was started, we had fully decided to use ether for anæsthesia and to determine in some special anæsthesia experiments the metabolic effects of this substance. During the progress of the first experiment we saw, however, that the time at our disposal for this work in collaboration would not be sufficient to enable us to study in detail the metabolic effects of the anæsthetic. Consequently, our observations on the effects of ether anæsthesia were confined to the dogs used in the hemorrhage experiments. Facts in this connection were noted merely for the purpose of checking the results of the conditions associated with the blood-letting.

Dogs are not quickly anæsthetized with ether, and usually struggle violently before they are overcome. Chloroform acts rapidly. Chloroform-ether mixture was accordingly administered at the beginning of each period of anæsthesia in order to diminish the initial period of resistance and excitement, and to reduce the metabolic effects due to such influences. Because of its vigorous catabolic action, however, only a trifling quantity of chloroform was taken for this purpose.

A number of writers have stated recently that "the changes in the metabolism following the use of chloroform (chiefly catabolic effects, such as increased nitrogenous excretion) are not produced to the same extent, if at all, by ether."<sup>2</sup> The work of various observers, however, has shown marked metabolic influences on the blood and urine after ether narcosis, but the extent of corpuscular disintegration and

<sup>1</sup> Exceptions due to hemorrhage are referred to on pages 185, 186.

<sup>2</sup> CUSHNY: *Text-book of Pharmacology and Therapeutics*, 1902 (2d ed.), p. 164.

of leucocytosis, or degree of albuminuria or glycosuria, for example, appear to depend chiefly on the depth of the anæsthesia and on the length of the period of its continuation. Anæsthesia was kept as light as possible in all our experiments, and, with the exception of slight transitory glycosuria, no abnormality of the urines was observed (see page 206).

Our knowledge of the effects of ether on proteid metabolism needs extension. That ether seems to stimulate slightly nitrogenous catabolism is suggested by the results of such experiments as those by Leppmann<sup>1</sup> and Taniguti;<sup>2</sup> but Zaleski's<sup>3</sup> work, on the other hand, indicates the opposite.<sup>4</sup> We have not depended on any previous statements in this connection, but have checked the influence of the anæsthetic on each dog subjected to acute anæmia.<sup>5</sup>

All blood-letting in these experiments was conducted from the femoral artery or a branch of it. In some cases the least possible disturbance of local circulation was sought, in other instances relatively great interference was intended. These ends seemed best attainable by opening either a very small artery or a large one. Further, such procedure gave results similar to those after relatively small hemorrhages, or after the largest and most troublesome losses.

The operations were conducted under aseptic conditions. The incisions were short and only of a superficial character. The loss of blood due to the operation itself was always less than 3 c.c. The wounds healed rapidly with only very slight, sometimes scarcely any, serous exudation, and with little or no observable inconvenience to the animal. Even when the femoral artery itself was ligatured, the temperature of the limb soon returned to the normal. In our third experiment, in which blood was drawn thrice from one femoral artery at intervals of a few days (see page 226), the dog did not appear to be inconvenienced in the least. It seemed to use each

<sup>1</sup> LEPPMANN: *Mitteilungen aus den Grenzgebieten der Medizin und Chirurgie*, 1899, iv, p. 37.

<sup>2</sup> TANIGUTI: *Archiv für pathologische Anatomie und Physiologie*, 1890, cxx, p. 123.

<sup>3</sup> ZALESKI: *Berichte der deutschen Botanischen Gesellschaft*, 1900, xviii, p. 292. See also Schipilin: *Jahresbericht der Thier-chemie*, 1892, xxii, p. 409.

<sup>4</sup> Dr. HAWK is now investigating this problem in the laboratory of Physiological Chemistry at the University of Pennsylvania. See *Proceedings of the American Physiological Society*, *This journal*, 1904, x, p. xxxvii.

<sup>5</sup> For further reference to these matters, see pages 181, 182, 194, 201, 208, 212, 216, 218, 227.

leg as freely immediately after operation as it did before. Care was always taken to prevent harm to exposed nerve-trunks. At the conclusion of each hemorrhage the severed artery was carefully ligatured and the lips of the wound were sutured. The dog's nose always remained moist and cold after the surgical operations.

When blood was drawn, it was directed through a cannula connected with a short rubber tube leading into a beaker containing a moderate excess (though known volume) of 25 per cent solution of sodium chloride. The combined weight of the beaker and the saline solution was taken, also the weight with the added blood. The weight of the blood was determined by difference. The saline solution served the purpose of keeping the blood in fluid condition for analysis. Weighed amounts of the mixture were taken for this purpose.

As already stated, our days of experiment ended at 5 P. M., and our surgical procedures were concluded before 10 A. M. About two-thirds of the day of hemorrhage had passed, therefore, before blood was drawn, but on this schedule there was a long period, of about fourteen hours, following each hemorrhage, during which we could conveniently observe any initial effects.

The influences of the anæsthetics and of our usual surgical operations were determined independently on each animal in order to check, in each instance, the results following blood-letting under anæsthesia.

*Collection of excreta.*—We collected the urine as it passed from the body normally. The periods were of sufficient length for the equalization of diurnal variations, thus making catheterization unnecessary. Then, too, these long periods were always purposely brought to an end on days on which the dog happened to urinate at about the closing hour—5 P. M. In this way we further insured period-neutralization of diurnal fluctuations in excreted volume.<sup>1</sup> Samples of the daily urine were immediately analyzed and a composite sample of the entire urine of each period was also made up for further chemical examination, and to check the total of daily results.<sup>2</sup> All urine not immediately analyzed was preserved in stoppered bottles with powdered thymol.

The addition of bone ash<sup>3</sup> to the diet increased the bulk of the

<sup>1</sup> See further references to period-equalizations, on page 198.

<sup>2</sup> Results showing the accuracy of both sets of analytic results are given on page 198.

<sup>3</sup> The amounts we used are indicated on pages 180, 208. See GIES: Proceedings of the American Physiological Society, This journal, 1904, x, p. xxii.

faecal matter and made its discharge more frequent and regular. These facts and the length of the periods made it unnecessary to introduce with the food a specially indigestible material to mark off the faeces at the end of a period. The faeces had the typical consistency and appearance of the excrementitious matter eliminated from dogs subsisting on a diet containing bone. There never was any diarrhoea, the faecal matter did not stick to the cage, it dried easily and could be readily reduced to a light homogeneous powder. Therefore there was no mixing of fluid faecal matter with urine, as is otherwise so often the case. In all instances the faecal matter was immediately dried on the water bath, and the fractions collected during each period were finally united, powdered, and bottled for analysis.

We wish to emphasize the fact to which attention has already been drawn,<sup>1</sup> that in equilibrium experiments on dogs the cast-off hair and dandruff must be carefully preserved and analyzed. In these experiments such offal was each day removed from the cage, in which practically all of it had lodged, and the combined lot for each period was subjected to analysis. A dog usually removes more or less hair from his body with his teeth and tongue. Much of the hair removed in this way appears in the faeces. In our experiments the faecal hair was separated from the powdered faeces with a very minutely meshed sieve and united with the main portion collected.<sup>2</sup> The bone ash favored this process by facilitating the pulverization of the mixture.

At the end of each period the cage was thoroughly washed and the combined washings analyzed. The small volume of urine adhering to the inclined bottom of the cage after the elimination of each fraction soon dried without undergoing chemical alteration. The attendant loss from the daily volume was very slight, but the dissolved constituents were, of course, completely recovered in the washings.<sup>3</sup> To these washings was added such saliva as passed from the animal during anaesthesia.

*Analytic methods.*—The ingredients of the food and the various excreta were subjected to analytic methods. All determinations of nitrogen were made by the Kjeldahl process. Oxidation was effected with concentrated sulphuric acid, aided by a little cupric

<sup>1</sup> MEAD and GIES: *Loc. cit.*

<sup>2</sup> The trifling quantity of hair washed from the bottom of the cage into the urine was obtained by filtration.

<sup>3</sup> Analytic data in this connection are given on page 200.

sulphate.<sup>1</sup> Total sulphur and phosphorus were determined by the well-known fusion processes. Chlorine was estimated by Mohr's method.<sup>2</sup> Approximate specific gravity of the urine and blood was ascertained with a urinometer. Total solids in the urine were calculated, from the figures for specific gravity, with the aid of Long's factor.<sup>3</sup> The quantities of material used for each analysis were those customarily employed. The purity of our reagents was always established before their use. The recorded analytic data are averages of closely agreeing duplicate results.<sup>4</sup>

**Experiments on the flow of urine after hemorrhage.** — Reference to these experiments may be found on pages 228.

### III. FIRST METABOLISM EXPERIMENT. — INFLUENCE OF HEMORRHAGE ON PROTEID CATABOLISM.

The animal used in this experiment was a plump, vigorous, short-haired dog weighing a little over 17 kilos.

**Diet.** — The character of the diet was the same throughout the whole experiment, although it varied slightly in quantitative composition, as indicated in Table I, at the top of the following page.<sup>5</sup>

<sup>1</sup> MARCUSE: *Archiv für die gesammte Physiologie*, 1896, lxiv, p. 232. The digestions in concentrated sulphuric acid were continued for several hours after the mixtures had become colorless.

<sup>2</sup> NEUBAUER and VOGEL: *Harnanalyse* (NEUBAUER and SALKOWSKI'S modification), 1898, p. 709.

<sup>3</sup> LONG: *Journal of the American Chemical Society*, 1903, xxv, p. 262.

<sup>4</sup> Other general matters relating to the conduct of these experiments were carried out as in the investigations by MEAD and GIES: *Loc. cit.*

<sup>5</sup> In the original description of the method used for preparing and preserving the meat fed during these experiments (GIES: *This journal*, 1901, v, p. 235; *Biochemical researches*, 1903, i, p. 69), it was shown that the products of such preparation, even at wide intervals and of different samples of beef, prove to be very uniform in composition. At that time, however, only the results of nitrogen determinations had been obtained. The following average data from the records of these experiments further emphasize the original deductions in this connection.

PERCENTAGE-COMPOSITION OF SAMPLES OF PREPARED HASHED BEEF.

Preparation.	Nitrogen.	Sulphur.	Phosphorus.
1	3.67 <sup>1</sup>	0.279	0.223
2	3.58	0.302	0.218
3	3.81	0.251	0.218
4	3.76	0.288	0.220

<sup>1</sup> All of the figures are averages of closely agreeing duplicate results.

TABLE I.  
COMPOSITION OF THE DAILY DIET.

Ingredients.	Hashed lean beef.			Cracker meal.	Lard.	Bone ash.	Water.
Days . . . . .	1-33	34-62	63-85	1-85			
Daily amounts <sup>1</sup>	grams 250.0	grams 256.4	grams 241.0	grams 70.0	grams 30.0	grams 10.0	c.c. 500.0
Nitrogen . . . .	9.175	9.175	9.175	1.085	0.008	0.003	....
Sulphur . . . .	0.698	0.775	0.606	0.092	0.009	0.006	....
Phosphorus . . .	0.557	0.558	0.527	0.094	0.026	1.779	....

TOTAL COMPOSITION OF EACH DAILY MIXTURE.				
Days.	Total weight.	Nitrogen.	Sulphur.	Phosphorus.
	grams	grams	grams	grams
1-33	860.0	10.271	0.805	2.456
34-62	866.4	10.271	0.882	2.457
63-85	851.0	10.271	0.713	2.426

<sup>1</sup> Three different preparations of meat were used. These varied slightly in composition. The nitrogen-content was 3.67, 3.58, and 3.81 per cent respectively. In order to keep the nitrogen-content of the daily food exactly the same throughout the experiment, the slight variations in weight of meat taken were necessitated. The variations in content of water, proteid, etc., induced in this way were immaterial.

**Preparatory period.**—A preparatory period of four days on the above diet sufficed to accustom the dog to the new food and environment. The weight of the animal during this time fell from 17.23 kilos to 17.02 kilos. The preparatory period was terminated on November 1, 1902, and on that day, at 5 P. M., the collection of analytic data was begun. The experiment continued without interruption until the night of January 24, 1903 (eighty-fifth day), and was divided into eight periods of varying length and different conditions, as follows:—

**First period. Normal conditions. Maintenance of nitrogenous equilibrium.** Days, 1-12; November 1-12, 1902.—Approximate equilibrium was established without special difficulty, and the animal was further accustomed to its new environment.

**Second period. Combined effects of anæsthesia, operation, and slow hemorrhage. Days, 13-28; November 13-28.**—On the thirteenth day the first blood was withdrawn. We desired to bring about the first hemorrhage with only slight injury to the tissue at the point of incision, and with as little disturbance of the local circulation as possible. We concluded that one of the smaller branches of the femoral artery would offer the greatest advantages in these respects. Accordingly, the dorsal ramus of the saphenous branch of the femoral artery in the right leg was selected for this purpose, but we found it impossible to force our smallest cannula into it. This delay in our procedure was regretted, because it had been our desire to make the period of anæsthesia as brief as possible. We then quickly made another short incision, a little higher up, and finally inserted the cannula into the saphenous artery itself.

After the hemorrhage had been started, coagula in the tube attached to the cannula occasionally stopped the flow into the beaker, but new tubes were used when necessary. The hemorrhage was quite slow to the end because of the smallness of the outlet and the associated clotting tendency.

*Schedule of operations.*—8.10 A.M., anæsthesia begun. 8.45, first incision made. 9.05, second incision. 9.26, hemorrhage started. 10.14, hemorrhage concluded. 10.20, sutures in place and anæsthetic discontinued. 10.55, dog sat up.

*Blood.*—The amount of blood withdrawn was 492.9 grams, a quantity equal to 2.93 per cent of the weight of the dog at the end of the twelfth day.

**Third period. Effects of anæsthesia alone. Days, 29-38; November 29-December 8.**—An initial increase in nitrogen output had been observed during the previous period (Tables II and VI), but toward the end of the period the dog returned to nitrogenous equilibrium, and seemed to have entirely recovered from the effects of the hemorrhage. It was impossible, of course, to say which one, or how many, of the three conditions attending the first hemorrhage effected the increased nitrogenous excretion. The known effects of chloroform and ether led us to think that a part, if not all, of the observed effect was due to the anæsthetics alone. Then, too, the healing process and the changes in the limb, with its lessened blood-supply, may have contributed a share in the observed results (see page 224).

In this period and the one following it, we therefore made an effort to determine the effects of anæsthesia, and also of the operation on this particular dog, in order to arrive by difference, if possible, at the immediate effects of the hemorrhage itself. Consequently, on the twenty-ninth day the animal was subjected to the same anæsthetics, at the same time of day, for the same period of time, and to the same degree, as on the day

of the first hemorrhage.<sup>1</sup> After ten days, when the dog again appeared to be in an entirely normal condition, the fourth period was begun.

**Fourth period. Combined effects of anæsthesia and operation.<sup>2</sup> Days, 39-51; December 9-21.** — The combined influence of the three conditions — anæsthesia, operation, and hemorrhage — was determined in the second period. The effect of anæsthesia alone was ascertained in the preceding period. In that period, in spite of an increase in the volume of the urine after the anæsthesia, there was a slight initial decrease in the elimination of nitrogen, the reverse of the effect observed when all three conditions were acting together (Tables II and VI). Thus it appeared that the effects of the anæsthesia had been opposed to the effects of the hemorrhage and the operation.<sup>3</sup> It now remained for us to ascertain the influence of the operation, and also whether the results noted in the second period were due to the operation, or to the loss of blood, or to both conditions. This was done indirectly by combining anæsthesia and operation, and comparing with former results.

The operation and anæsthesia were exactly the same as for the hemorrhage at the beginning of the second period. On the thirty-ninth day, when the dog was again in approximate nitrogenous equilibrium, two incisions were made in the same regions, on the opposite leg (left), however. The same small artery and its branch were ligatured and severed. All conditions, except loss of blood, were practically the same, in time and degree, as at the operation of the second period.

**Fifth period. Effects of a second and rapid hemorrhage. Days, 52-72; December 22, 1902-January 11, 1903.** — The influence of the anæsthesia and operation on nitrogenous elimination in the last period, was almost as great as in the first hemorrhage period (Tables II and VI).

<sup>1</sup> About 5 c.c. of urine was suddenly ejected during the initial administration of the anæsthetics. It was collected with filter paper and added to the cage-washings. There were only two additional occurrences of this kind in all our experiments. See pages 184 and 185.

<sup>2</sup> By the term "operation," we mean not only the incision but the ligation of the blood-vessels, suturing of the wounds, etc.

<sup>3</sup> It must be admitted, of course, that although we were dealing with the same animal throughout this experiment, the plane of metabolism was at a different level in each period, and that, therefore, our "checks" are only approximate. But in this particular connection our anæsthesia results are relatively none the less significant, for the weight of the dog was lower at this time than before, and, although his food was the same, he gained in weight from the beginning of the period. After hemorrhage there was a permanent fall off in weight, except in one instance, even during the later periods of the experiments, when the animal was in need of still more constructive material (see Table II). It is hardly probable that the effects of ether in this connection are *reversed* after a hemorrhage. See page 218.



This fact makes it seem probable that the first hemorrhage was without very decided influence on the catabolism of proteid matter, and that the observed effect in the second period was largely due to the operation, or the healing process, or some associated factor (page 224), although it is possible that the usual conditions throughout the preceding period were no longer entirely comparable to those of the second.<sup>1</sup> That they were not strictly comparable in the middle of that period is indicated by the facts referred to on page 188.

We next attempted to ascertain the influence of several additional hemorrhages, and, at the same time, to further check, if possible, the results of the second period.

The second blood-letting was effected on the fifty-second day, at the usual time, under conditions essentially the same as on the thirteenth day, except that the blood was *rapidly* removed, the operation was performed on the left leg (same as in the fourth period), and the blood was drawn directly from the femoral artery, just above the point where the saphenous branch left it. A single incision was made, which was a trifle longer than the ones before, because of failure to insert the cannula into the remaining portion of the saphenous artery, but it was about equal in length to the two short incisions in each of the second and fourth periods.

*Schedule of operations.* — 8.14 A. M., anæsthesia begun. 8.40, first incision made. 9.12, second incision. 9.31, hemorrhage commenced. 9.40, hemorrhage concluded. 10.05, anæsthetic discontinued. 10.10, sutures in place. 10.45, dog sat up.

*Blood.* — The amount of blood removed was 5.06 grams, a quantity equal to 3.22 per cent of the weight of the dog at the end of the fifty-first day, or 3.01 per cent of body-weight at the end of the twelfth day.

**Sixth period. Effects of a third (rapid) hemorrhage. Days, 73-79; January 12-18.** — The effects of the second hemorrhage were more decided than any catabolic results noticed previously (Tables II and VI). Although the dog had practically returned to the normal vascular condition, it was evident that the second drain on the system, with the associated operation, etc., had exerted a more marked influence than the first. This effect could not have been due, to any great extent, to the healing process, as was the case, perhaps, in the second period, although a greater disturbance in the circulation of the limb may have been a contributing factor.<sup>2</sup> The amount of blood lost during this hem-

<sup>1</sup> A puzzling fact in this connection was the steady increase in the weight of the dog in spite of the increased loss of nitrogen. After hemorrhage, the weight of the dog continued to fall in all cases but one, in which instance, it remained stationary. See page 192.

<sup>2</sup> In effecting the fourth hemorrhage, however, the disturbance of circulation was just as marked, but on the opposite side, yet the catabolic effects were greater, as was shown in a more decided increase of nitrogen in the urine.

orrhage was only slightly greater than that of the first.<sup>1</sup> After waiting three weeks for restoration of nitrogenous equilibrium, and practically complete recovery, a third hemorrhage was effected.

The usual conditions prevailed for this third hemorrhage. Blood was withdrawn from the femoral artery which had been opened in the previous period. The cannula was inserted as near the former opening as possible. Lateral branches were not permanently disturbed. The blood was withdrawn rapidly. The immediate effects of this hemorrhage became so evident, in almost imperceptible pulse and retarded respiration, for example, that the anæsthetic was discontinued before the hemorrhage was stopped, and the ligatures and sutures were attended to as rapidly as possible, while the animal was recovering from anæsthesia. Recovery was more prompt than we expected.

*Schedule of operations.* — 8.20 A. M. anæsthesia begun. 8.41, operation commenced. 9.15, hemorrhage started. 9.20, anæsthetic discontinued. 9.24, blood-letting stopped. 9.52, sutures in place. 10.20, dog sat up.

*Blood.* — The amount of blood withdrawn was 505.5 grams, a quantity equal to 3.51 per cent of the weight of the dog at the end of the seventy-second day, or 3.01 per cent of body-weight at the end of the twelfth day.

**Seventh period. Effects of a fourth (slow) hemorrhage. Days, 80–83 ;**

**January 19–22.** — The metabolic effects of the third hemorrhage were similar to those after the second period of anæmia, but were not so pronounced, even though the relative amount of blood abstracted was greater than any quantity previously taken (Tables II and VI). The blood was removed from the same vessel, however, and disturbance of circulation in the limb in this period was not increased beyond what it had been. Consequently there may have been less influence of the surgery this time (see page 209).

We concluded to remove blood again, and in large volume, before the dog could fully recover from the effects of the previous loss. Under such conditions it might be presumed that the combined catabolic effects of operation and anæsthesia would be *relatively* less if acute anæmia itself showed cumulative catabolic action.

Blood was taken from the right femoral, just above the point at which the saphenous branch left it, and without disturbing any of its lateral ramifications. After about 400 grams of blood had been drawn, the flow became very slow, because of persistent clotting within the cannula. In spite of repeated removals of clots the blood issued from the tube drop by drop, rather than in spurts, as before. Although heart-beat was

<sup>1</sup> There had been sufficient time for full regeneration of the blood.

very weak, and respiration much slower, we continued the hemorrhage until 444.5 grams of blood had been removed. The administration of the anæsthetic was discontinued a few minutes earlier than at the first two hemorrhages, just as in the sixth period.

Recovery was fairly rapid, though not as prompt as before. For the first time the dog failed to show an eagerness for his food. The weight of the mixed diet was 851 grams. Of this the animal ate 362 grams at the usual time, slowly and indifferently. The dog seemed to tire of standing. A few minutes later the rest of the food was offered to the animal while it was lying down. All of it was slowly taken. All was retained. The dog's strength and appetite had, however, been markedly affected. Even on the following day the effect on the appetite was still evident, although all the food was gradually eaten.

*Schedule of operations.* — 8.29 A.M., anæsthesia begun. 8.55, operation commenced. 9.15, about 50 c.c. urine eliminated and caught. 9.16, respiration, 41. 9.18, hemorrhage started. 9.21, 100 grams blood obtained. 9.23, 200 grams blood. 9.25, 300 grams blood. 9.29, 400 grams blood. 9.39, respiration, 22. 10.18, hemorrhage stopped. 10.19, respiration, 30; anæsthetic discontinued. 10.31, stitches in place. 10.56, dog sat up.

*Blood.* — The amount of blood withdrawn was 449.3 grams, a quantity equal to 3.22 per cent of the weight of the dog at the end of the seventy-ninth day, or 2.67 per cent of body-weight at the end of the twelfth day.

**Eighth period. Effects of a fifth, and fatal hemorrhage. Days, 84–85 ;**

**January 23–24.** — In the previous period we saw the most pronounced after-effects of hemorrhage, also the highest degree of catabolism, as shown in greatly increased output of nitrogen, in spite of the severe shock to which the animal was subjected (Tables II and VI). The results seemed to indicate that after *excessive* hemorrhage proteid catabolism was relatively so markedly stimulated that the possible effects of the operation and anæsthesia could hardly account for the observed increase.

Although the previous hemorrhage probably gave us maximum catabolic results, we ran the risk of causing death, in order, a few days later, to bring out whatever additional effects might be shown after another loss of blood. Unfortunately, the last removal was too much for the dog, but the cumulative effect of further hemorrhage was distinctly shown.

The last hemorrhage was conducted under conditions practically identical with those of the preceding period, except that the new incision was made a little higher on the same femoral artery, and with the occlusion of only one additional lateral branch. A short cylindrical clot had to be removed from the artery before the cannula could be inserted. Blood was drawn until death again seemed imminent, as indicated by gasping, almost imperceptible heart-beat, spontaneous elimination of a small quan-

tity of urine (which was completely caught), rigidity of the tail, etc. The flow of blood was very slow at this point, and very troublesome in its clotting tendency. The mixture of blood and excess of strong saline solution (274 grams of 25 per cent NaCl) showed a semi-gelatinous consistency after a few minutes, and contained considerable fibrin and other white masses (leucocytes). Administration of the anæsthetic was stopped early, as in the two previous periods.

Consciousness soon returned, but the animal showed no desire to stand or sit up. At noon the pulse was rapid and weak, respiration slow and shallow. The temperature of the body gradually fell. Food was entirely refused at 5 p.m. and thereafter. In spite of the use of a hot-water bag, blankets, and other devices to keep up its temperature, the animal did not revive. Death occurred at 7 o'clock. There were no convulsions.

*Schedule of operations.* — 8.30 A.M., anæsthesia begun. 8.42, operation started. 8.52, hemorrhage commenced. 9.19, hemorrhage and anæsthetic stopped. 9.54, stitching finished. No attempt at any time to rise.

*Blood.* — The amount of blood withdrawn was 317.5 grams, a quantity equal to 2.46 per cent of the weight of the dog at the end of the eighty-third day, or 1.89 per cent of body-weight at the end of the twelfth day.

As has already been indicated, a small quantity of urine was passed during the operation. The dog urinated again at 1 o'clock in the afternoon — 152 c.c.; specific gravity, 1026. The urine in the bladder at the time of death, six hours later, amounted to only 14 c.c., and had the highest specific gravity of any sample yet obtained — 1036. Post mortem examination indicated very complete and general exsanguination. The only blood to be found in appreciable quantity in the heart was a small clot in the left ventricle.

The "blood count" in samples of the blood which was removed in the earlier stages of the hemorrhage indicated a very marked decrease in the number of red corpuscles per cubic millimetre. There were so many clusters of colorless cells that an accurate estimation was impossible, but they were enormously increased in number.<sup>1</sup> They were very conspicuous in all parts of every field examined. Even the outward appearance of the blood indicated the presence of an abnormal number of leucocytes and a diminished number of erythrocytes. Of course, these facts show the condition of the blood at the *end of the last period*. After the hemorrhage of this period the residual blood was naturally still more abnormal. Throughout the entire experiment each hemorrhage increased the tendencies in these directions.

<sup>1</sup> Leucocytes were in excess of the number seen in post-operative, non-septic leucocytosis. See KING: American journal of the medical sciences, 1902, cxxiv, p. 450.

The results of the last hemorrhage are of value chiefly in showing how near we were to a fatal termination after the former one. The results following the previous loss of blood doubtless represent the maximum catabolic effects of excessive hemorrhagic anæmia.

**Supplementary data, Periods I-VIII.**—In addition to the foregoing facts the following observations were also made.

The fluctuations in the temperature of the room were between 17° C. and 25° C. The temperature was usually about 21°–22° C.

Respiration and blood-pressure appeared to depend on the quantity of blood removed, on the rate of its withdrawal, and on the length of time after hemorrhage.<sup>1</sup> Variations in respiration were frequent.

The direct losses in weight on the day of operation varied with the procedure. The amount of blood drawn, the volume of saliva secreted under the influence of the anæsthetic, and the volume of the urine excreted, seemed to be the chief factors in this connection.

The reaction of the urine was usually acid to litmus. After the anæsthesia alone, and the operation and anæsthesia combined, the reaction was distinctly, though only slightly, amphoteric, but soon became decidedly acid again. After the hemorrhages the reaction was always persistently amphoteric, the alkalinity sometimes almost equalling the acidity, and the urines showing turbidity due to earthy phosphate. The amphoteric reaction gradually diminished, and eventually gave way to the normal acidity.

The effect of hemorrhage on hypersecretion of saliva under the influence of the ether was very marked. The flow of saliva was greatest when only the anæsthetic was administered, or when the anæsthetic and operation were combined. It became progressively less with each hemorrhage. At the last two blood-lettings there was practically no salivation associated with the administration of the anæsthetic (see page 223).

Edema about the point of operation was observed on only one occasion. This was on the fifty-fourth day. The swelling extended for a short distance on the abdomen, along the penis, on the side of the operation. All signs of it were gone on the following day.

The blood became thinner and thinner with each hemorrhage, and at the last operation had an appearance approaching that of lakiness (page 220). Most of the red cells seemed to be a little larger in the last sample of blood than in any before, just as though they had become somewhat swollen.<sup>2</sup> Proteid was much diminished, but inorganic content was essentially unchanged. Progressive tendency to coagulate was also observed, culminating in the striking effect, at the last hemorrhage, noted above.

<sup>1</sup> Our data confirm those of DAWSON: This journal, 1900, iv, p. 6.

<sup>2</sup> Similar facts were also observed by WILLEBRAND.

None of the wounds could be successfully kept bandaged. Bandaging was tried at various times, but the dog was determined to remove everything placed upon him. Fearing that such efforts would do more harm than any good the bandages could accomplish, we permitted the wounds to be exposed to the dog's own attention.<sup>1</sup> On the forty-fifth day, after an attempt to bandage the wound because of the accidental hemorrhage referred to below, the animal removed and swallowed some strips of adhesive plaster. The pieces of the plaster began to appear in the feces on the forty-sixth day, and passed promptly through the gastro-enteric tract without exerting any apparent deleterious effect. The increase in the amount of feces at that time (forty-sixth day), exclusive of the weight of the bandage thrown out, indicated a disturbance of digestion. But the incidents referred to below also contributed a share in vitiating our results during the middle of this period.

On three occasions there were accidental hemorrhages from the wounds. A few drops of blood were lost on the fifteenth day from a superficial source, when a stitch tore through the edge of one lip of the wound. On the eighty-third day there was a similar insignificant loss. On the forty-fifth day, however, six days after the last previous operation, when the wound seemed to be healing rapidly, a hemorrhage suddenly occurred which threatened to end our experiment. At 1 P. M. on that day the dog was heard to move suddenly, and the sound of rapid lapping further attracted our notice. Instant attention was given the matter, and the dog was found to be lapping a small, though strong and persistent current of blood from the wound. A few drops escaped to the bottom of the cage, but the rest of it had been swallowed before we were able to stop the flow. We do not believe that more than 25 c.c. of blood could have passed from the wound in this interval. Pressure on the wound was continued for some time, and the small clot in the opening and under the remaining stitches was allowed to stay in place. The dog was kept in a recumbent position for an hour or two, until immediate danger of recurrent hemorrhage had passed. The appearance of the wounds before and after this incident made it probable that this was the only special accident of the kind, although a few new drops of blood on the bottom of the cage on the following morning suggested that a loss had occurred again during the night. The appearance of the wound made it certain, however, that this second loss must have been inconsiderable, for the old clot was still in place. It was during these few days that the increased catabolism of the fourth period was relatively conspicuous.<sup>2</sup>

<sup>1</sup> References to the probable effects, on metabolism, of licking the wound may be found on page 223.

<sup>2</sup> The feces at this time were darker in color because of the swallowed blood.

TABLE II.

DAILY RECORDS OF THE FIRST METABOLISM EXPERIMENT.<sup>1</sup>

I. First period. Maintenance of normal nitrogenous equilibrium. Nov. 1-12, 1902.								
DAY. No.	BODY- WEIGHT.	URINE.					FÆCES. <sup>2</sup>	
		Volume.	Specific gravity.	Nitrogen.	Period av'ge to date.		Weight.	
					Volume.	Nitrogen.	Fresh.	Dry.
	kilos	c.c.		grams	c.c.	grams	grams	grams
1	16.96	499	1017	8.30	499	8.30	46.5	24.4
2	16.96	581	1019	10.23	540	9.26	....	....
3	16.96	394	1021	7.82	491	8.78	43.1	22.1
4	16.95	499	1020	9.84	493	9.05	35.8	19.7
5	16.92	461	1020	8.56	487	8.95	27.8	15.6
6	16.89	445	1020	8.10	480	8.81	71.1	36.4
7	17.01	400	1018	6.51	468	8.48	....	....
8	16.78	662	1018	11.27	493	8.83	54.1	38.1
9	16.91	436	1018	7.33	486	8.66	32.4	18.2
10	16.83	443	1019	7.71	482	8.57	30.6	17.7
11	16.85	486	1019	8.57	482	8.57	32.3	17.8
12	16.80	560	1019	10.60	489	8.74	27.2	14.4

II. Second period. Combined effects of anæsthesia, operation, and hemorrhage (2.93%). November 13-28.								
13	16.29	377	1017	6.33	377	6.33	34.3	20.2
14	16.20	556	1027	13.34	467	9.84	....	....
15	16.20	501	1022	10.87	478	10.18	36.1	20.5
16	16.25	370	1022	7.63	451	9.54	69.1	34.1
17	16.20	570	1025	13.67	475	10.37	....	....
18	16.32	307	1017	5.25	447	9.51	45.3	23.1
19	16.37	475	1022	9.84	451	9.56	33.6	20.0

<sup>1</sup>	Facts regarding food are given in Table I.
<sup>2</sup>	Blanks under fæces in this and all subsequent tables signify no elimination.

TABLE II — *continued.*

II. Second period — <i>continued.</i>								
DAY. No.	BODY- WEIGHT.	URINE.					FÆCES.	
		Volume.	Specific gravity.	Nitrogen.	Period av'ge to date.		Weight.	
					Volume.	Nitrogen.	Fresh.	Dry.
	kilos	c.c.		grams	c.c.	grams	grams	grams
20	16.33	586	1021	11.40	468	9.79	38.2	19.0
21	16.34	462	1020	9.69	467	9.78	41.8	27.5
22	16.33	512	1019	8.84	472	9.69	....	....
23	16.32	520	1018	8.20	476	9.55	37.5	19.5
24	16.28	498	1019	9.41	478	9.54	37.1	20.7
25	16.29	463	1021	9.28	477	9.52	35.8	17.4
26	16.27	444	1019	7.56	474	9.38	42.5	21.6
27	16.18	576	1019	10.33	481	9.44	40.3	20.0
28	16.09	615	1020	11.04	489	9.54	32.8	16.9
III. Third period. Effects of anæsthesia alone. November 29–December 8.								
29	15.81	654	1018	8.59	654	8.59	64.0	35.9
30	15.85	495	1021	8.56	575	8.58	....	....
31	15.88	467	1019	8.80	539	8.65	36.2	20.1
32	16.02	388	1021	8.22	501	8.54	31.3	16.7
33	16.03	512	1020	10.70	503	8.97	59.3	32.6
34	16.09	458	1019	8.06	496	8.82	....	....
35	16.00	570	1018	9.01	506	8.85	64.0	36.4
36	16.00	555	1018	8.78	512	8.84	32.6	17.3
37	15.98	593	1017	9.20	521	8.88	....	....
38	15.94	532	1020	9.65	522	8.96	71.9	35.3
IV. Fourth period. Combined effects of anæsthesia and operation. December 9–21.								
39	15.44	754	1018	9.98	754	9.98	40.0	20.5
40	15.42	572	1020	10.72	663	10.35	....	....



TABLE II — *continued.*

IV. Fourth period — <i>continued.</i>								
DAY. No.	BODY- WEIGHT.	URINE.					FÆCES.	
		Volume.	Specific gravity.	Nitrogen.	Period av'ge to date.		Weight.	
					Volume.	Nitrogen.	Fresh.	Dry.
	kilos	c.c.		grams	c.c.	grams	grams	grams
41	15.64	330	1019	6.73	552	9.15	35.2	19.0
42	15.67	520	1024	12.28	544	9.93	28.1	15.5
43	15.68	530	1020	10.07	541	9.96	63.4	35.4
44	15.68	596	1018	9.27	550	9.84	....	....
45	15.72	400	1019	7.77	529	9.55	41.9	21.7
46	15.69	558	1020	11.12	533	9.75	118.8	51.4
47	15.68	526	1023	12.81	532	10.09	....	....
48	15.66	556	1021	10.87	534	10.16	21.3	15.7
49	15.74	405	1018	7.41	522	9.91	32.5	15.7
50	15.70	575	1018	9.68	527	9.89	51.0	29.3
51	15.72	580	1018	10.00	531	9.90	23.2	13.3
V. Fifth period. Effects of a second hemorrhage (3.01%). December 22, 1902— January 11, 1903.								
52	15.18	428	1014	5.58	428	5.58	30.3	19.1
53	15.09	590	1027	13.51	509	9.55	....	....
54	14.92	579	1024	13.06	532	10.72	30.6	16.8
55	14.76	620	1020	11.62	554	10.94	57.8	37.7
56	14.76	478	1026	11.89	539	11.13	26.5	18.3
57	14.76	447	1024	10.27	524	10.99	18.4	11.1
58	14.77	534	1024	13.19	525	11.30	27.8	14.7
59	14.77	482	1022	10.93	520	11.26	41.5	20.7
60	14.78	415	1018	7.02	508	10.78	48.7	24.5
61	14.75	566	1023	11.77	514	10.88	24.3	16.7
62	14.70	598	1019	10.29	521	10.83	33.5	16.9
63	14.64	600	1020	11.47	528	10.88	31.8	16.8

TABLE II—*continued.*

V. Fifth period — <i>continued.</i>								
DAY. No.	BODY- WEIGHT.	URINE.					FÆCES.	
		Volume.	Specific gravity.	Nitrogen.	Period av'ge to date.		Weight.	
					Volume.	Nitrogen.	Fresh.	Dry.
	kilos	c.c.		grams	c.c.	grams	grams	grams
64	14.56	534	1020	10.33	529	10.84	69.0	37.7
65	14.44	620	1019	10.97	535	10.85	....	....
66	14.46	468	1020	9.48	531	10.76	35.5	17.5
67	14.45	546	1021	10.97	532	10.77	69.1	35.9
68	14.43	522	1019	9.61	531	10.70	....	....
69	14.45	510	1020	9.84	530	10.65	48.9	26.3
70	14.38	542	1019	9.72	531	10.61	33.5	21.0
71	14.40	518	1018	9.22	530	10.54	37.8	19.8
72	14.42	534	1018	9.17	530	10.47	103.8	46.1
VI. Sixth period. Effects of a third hemorrhage (3.51%). January 12-18.								
73	13.76	460	1018	7.91	460	7.91	....	....
74	13.80	475	1026	11.55	468	9.73	....	....
75	13.70	624	1021	13.01	520	10.82	47.5	23.0
76	13.76	394	1023	9.03	488	10.38	42.3	21.7
77	13.82	412	1024	10.44	473	10.39	57.8	28.0
78	13.80	484	1024	10.96	475	10.48	103.9	38.5
79	13.77	488	1018	8.33	477	10.18	56.5	19.7
VII. Seventh period. Effects of a fourth hemorrhage (3.22%). January 19-22.								
80	13.35	463	1018	8.88	463	8.88	....	....
81	13.36	518	1033	13.92	490	11.40	....	....
82	13.04	770	1022	17.14	584	13.31	29.5	15.9
83	12.90	464	1024	11.75	554	12.92	134.5	59.6

TABLE II—continued.

VIII. Eighth period. Effects of a fifth and fatal hemorrhage (2.46%). January 23-24.								
DAY. No.	BODY- WEIGHT.	URINE.					FÆCES.	
		Volume.	Specific gravity.	Nitrogen.	Period av'ge to date.		Weight.	
					Volume.	Nitrogen.	Fresh.	Dry.
84	kilos 12.63	c.c. 455	1026	grams 11.28	c.c. ....	grams ....	grams ....	grams ....
85	....	14	1036	Death occurred at the end of the second hour.				

IX. Supplementary data. Periods I-VIII. [See also Tables III-XI.]									
Blood and excretions.		PERIODS.							
		I. 12 days.	II. 16 days.	III. 10 days.	IV. 13 days.	V. 21 days.	VI. 7 days.	VII. 4 days.	VIII. 2 days.
		grams	grams	grams	grams	grams	grams	grams	grams
A. Urine:									
Total nitrogen . .		104.84	152.64	89.57	128.71	219.91	71.23	51.69	....
B. Cage washings:									
Nitrogen . . . .		1.83	2.39	1.97	2.54	3.44	0.95	0.58	....
Sulphur . . . .		0.17	0.25	0.15	0.19	0.39	0.13	0.10	....
Phosphorus . . .		0.19	0.22	0.17	0.20	0.31	0.99	0.06	....
C. Blood: <sup>1</sup>									
Specific gravity .		....	1063	....	....	1062	1058	1047	....
Percentage content:			per cent			per cent	per cent	per cent	per cent
Nitrogen . . . .		....	2.591	....	....	2.850	2.318	1.848	1.421
Sulphur . . . .		....	0.138	....	....	0.134	0.134	0.144	0.124
Phosphorus . . .		....	0.045	....	....	0.040	0.040	0.037	0.043

<sup>1</sup> The total amount of blood lost in the five hemorrhages was 2271.2 grams. The first four losses of blood amounted to 1953.7 grams, or 11.63 per cent of the weight of the dog on the day before the first hemorrhage.

**Analytic results.**—The more significant average results of daily and period duplicate analyses during this experiment are recorded in Tables II-XI.

**Discussion of results.** *Decline in weight.*—Examination of the data recorded in Table II shows that, in spite of the fact that the same amount of food was given daily, there was an irregular, though steady decline in the weight of the dog, a fall from 16.96 kilos at the beginning, to 12.63 kilos at the end. The greatest falls in weight occurred on the days of hemorrhage, with sometimes a rally during the next few days; but with the exception of the sixth period, there was

always a steady decline in weight after each loss of blood. Nitrogenous equilibrium was repeatedly re-established, but on successively lower planes. Loss of blood and saliva, increased catabolism, and greater excretion of water, are the more obvious reasons for these facts.<sup>1</sup>

*Fluctuations in volume of the urine.*—The daily volume of urine fluctuated between 307 c.c. (eighteenth day) and 770 c.c. (eighty-second day). The *average* daily quantity for each period gradually increased with the recurring blood-lettings (except after the third), in spite of the fact that the dog was evidently in need of water to replace that lost through hemorrhage and diuresis. The amount of water in the food remained the same each day.

TABLE III.  
TOTAL URINE VOLUMES ON THE OPENING DAYS OF THE PERIODS.

CONDITIONS.	PERIOD.	VOLUME OF URINE.			
	No.	First day.	Second day.	Third day.	Fourth day.
First hemorrhage . . .	II.	c.c. 377	c.c. <b>556</b>	c.c. 501	c.c. 370
Second hemorrhage . .	V.	428	590	579	<b>620</b>
Third hemorrhage . . .	VI.	460	475	<b>624</b>	394
Fourth hemorrhage . .	VII.	463	518	<b>770</b>	464
Anæsthesia alone . . .	III.	<b>654</b>	495	467	<b>388</b>
Anæsthesia and operation	IV.	<b>754</b>	572	<b>330</b>	520

<sup>1</sup> With each succeeding hemorrhage the low initial volume rose slightly, but the cumulative increase afterward became more decided and was longer continued. The effect of anæsthesia was similar in the first respect.

The figures for average volume tell only a part of the story, however. After each hemorrhage the volume for that day was always less than the daily average for the preceding period and for the period which it opened. In most cases the differences are very striking. The daily volume of urine after hemorrhage was *at first*, therefore, relatively small, but it rapidly increased to a *maximum* on the second or third day, and on one occasion (fifth period) the increase was

<sup>1</sup> See the third metabolism experiment for further facts in this connection.

cumulative to a climax on the fourth day. The results in the control periods, after anæsthesia alone, and following anæsthesia combined with operation, were just the reverse. On the days of anæsthesia the very highest volumes of the periods were attained, and there was an immediate and steady fall to a *minimum* on the third or fourth day.<sup>1</sup>

TABLE IV.

FRACTIONAL VOLUMES OF URINE ON THE OPENING DAYS OF THE PERIODS.

DAY OF EXPERIMENT. <sup>1</sup>		FRACTIONAL VOLUMES OF URINE.				
No.	Conditions.	Total vol. for the day. <sup>2</sup>	At 8 A.M. before operation.	S A.M. to 5 P.M.	Volume, and time of elimination, of the <b>first</b> fraction after operation (at 9 A.M.).	Volume, and time of elimination, of the <b>second</b> fraction after operation (at 9 A.M.).
13	1st hemorrhage	c.c. 377	c.c. 377	c.c. None	c.c. None till after midnight	c.c. None till after 8 A.M. next day
52	2d hemorrhage	428	428	None	None till after midnight	None till after 8 A.M. next day
73	3d hemorrhage	460	300 <sup>3</sup>	160	160 at 1.30 P.M. <sup>4</sup>	None till after 8 A.M. next day
80	4th hemorrhage	463	463 <sup>5</sup>	None	200 at 8.30 P.M. <sup>6</sup>	None till after 1 P.M. next day
84	5th hemorrhage	455	303 <sup>7</sup>	152	152 at 2 P.M. <sup>8</sup>	[Dog died at 7 P.M. —14 c.c.]
29	Anæsthesia alone	654	366	288	288 at 4.15 P.M.	170 c.c. at 9 P.M.
39	{ Anæsthesia and } { operation . . . }	754 <sup>9</sup>	460	294	294 at 4.20 P.M.	140 c.c. at 8.35 P.M.

<sup>1</sup> Ends at 5 P.M.  
<sup>2</sup> Note the increasing first-day volume.  
<sup>3</sup> This volume had been passed before the midnight preceding the operation.  
<sup>4</sup> This amount began to collect before the previous midnight. On other than hemorrhage days, the fractions were passed frequently.  
<sup>5</sup> Between 300–400 of this quantity had been eliminated about two hours before the previous midnight.  
<sup>6</sup> This volume began to collect shortly after the previous midnight.  
<sup>7</sup> Including the small volume (50 c.c.) passed during the operation, at about 9.15 A.M. See page 184.  
<sup>8</sup> This volume began to collect at 9.15 A.M., when presumably the bladder was emptied. See Note 7.  
<sup>9</sup> During the twenty-four hours from 8 A.M. of the thirty-ninth day to 8 A.M. of the fortieth day, 774 c.c. were passed—the largest volume for any twenty-four hours in the entire experiment.

<sup>1</sup> See Tables III and IV for further facts bearing on the effects of hemorrhage and ether on the excretion of urine, and page 228 for additional references to the influence of the former.

The initial retarding effects of hemorrhage on excreted volume are no more striking than the results of subsequent stimulation. Table III shows this with special clearness, and offers direct comparison with the opposite effects of the anæsthetic. In spite of the great stimulating effect of the ether, in the amounts used by us, on volume during the first twenty-four hours after anæsthesia alone, hemorrhage at once decreased the urinary excretion. Hemorrhage without anæsthesia would doubtless have given even more decidedly inhibitory results. These observations are further emphasized by the data in Table IV showing initial effects on fractional elimination.<sup>1</sup>

*Specific gravity of the urine.* — With only a few exceptions the specific gravity of the urine showed little variation from the average. On the day of hemorrhage there was always a decrease in specific gravity just as there was in volume. The specific gravity of the urine on this day always went to the lowest point recorded in the whole period, and was much lower than that of the preceding urines. On the following day, however, when the volume rose, specific gravity went up with it and, going to the opposite extreme, invariably proceeded to the highest mark registered in the period, falling shortly to the usual figure. Such effects were lacking in the control periods. These observations and the facts recited above make it probable that after a hemorrhage there is at least a temporarily increased total metabolism, in spite of the lessened resources of the blood still remaining in the body.<sup>2</sup>

*Nitrogenous catabolism.* — An examination of the many figures in Table II for nitrogen of the urine reveals the fact that a single moderate hemorrhage was followed by slight though only temporary stimulation of nitrogenous catabolism. Our data show that with the dog in approximate nitrogenous equilibrium before each blood-letting (except the last), there was an immediate increase in the output of nitrogen after each hemorrhage, this excess of excreted nitrogen being slight after the first hemorrhage, but growing somewhat larger with each successive loss of blood. That a minor part of this hemorrhagic stimulation of catabolism resulted each time from consequences of the surgical procedure itself seems to be indicated by the fact that there was a similar though smaller increase of nitrogen output during the anæsthesia-operation period (IV) and that, too, in spite of the fact

<sup>1</sup> On page 200 reference is made to the ratio between urinary nitrogen and volume. See also the special experiments on urine formation, page 228.

<sup>2</sup> Facts regarding total solids are given on page 203. See also page 201.

TABLE V.  
ANALYTIC TOTALS AND AVERAGES FOR NITROGEN IN EACH PERIOD.

	PERIODS.						
	I. 12 days.	II. 16 days.	III. 10 days.	IV. 13 days.	V. 21 days.	VI. 7 days.	VII. 4 days.
	grams	grams	grams	grams	grams	grams	grams
Food <sup>1</sup> . . . . .	123.25	164.34	102.71	133.52	215.69	71.90	41.08
Excreta . . . . .	116.05	167.30	99.10	140.71	239.03	77.13	55.25
Balance a . . . . .	+7.20	-2.96	+3.61	-7.19 <sup>2</sup>	-23.34	-5.23	-14.17
Blood . . . . .	....	-12.76	....	....	-14.45	-11.72	-8.30
Balance b . . . . .	....	-15.72	....	....	-37.79	-16.95	-22.47
AVERAGE DAILY BALANCES.							
Balance a . . . . .	+0.60	-0.19	+0.36	-0.55	-1.11	-0.75	-3.54
Balance b . . . . .	....	-0.98	....	....	-1.80	-2.42	-5.61
TOTAL NITROGEN OF EXCRETIONS.							
Urine and cage-washings . . . . .	106.67	155.07	91.54	131.25	223.35	72.18	52.27
Fæces . . . . .	7.09	8.96	5.81	7.55	12.32	3.94	2.43
Hair . . . . .	2.29	3.27	1.75	1.91	3.36	1.01	0.55
Total . . . . .	116.05	167.30	99.10	140.71	239.03	77.13	55.25
AVERAGE DAILY NITROGEN OF EXCRETIONS.							
Urine and cage-washings . . . . .	8.89	9.69	9.15	10.09	10.63	10.32	13.06
Fæces . . . . .	0.59	0.56	0.58	0.58	0.59	0.56	0.61
Hair . . . . .	0.19	0.20	0.18	0.15	0.16	0.14	0.14
Total . . . . .	9.67	10.45	9.91	10.82	11.38	11.02	13.81

<sup>1</sup> The average daily nitrogen of the food was **10.27** grams in each period.

<sup>2</sup> See discussion on page 188. See also Table VI.

that during the previous period, in which we determined the effect of anaesthesia alone, there was a distinct decrease in the excretion of nitrogen. That the increased nitrogen of the urine in this period (IV) cannot be ascribed to diuretic influences seems clear on comparing the results following the influence of ether alone (Period III), when diuresis was almost as marked, although nitrogen-output was actually *diminished*.<sup>1</sup>

Table V shows the differences indicated by the figures for totals and daily averages of nitrogen ingesta and excreta, etc., for the first seven periods of our experiment.

The data in Table V show only total and average effects. The "period averages to date," in Table II, help to make clear the initial effects of hemorrhage, etc., and they show, indirectly, the after-effects also. In long periods, such as those of the present experiments, there may be tendencies to equalization or submergence of initial effects; first results may be wholly or partly balanced by after-effects. This is especially true when the new condition marking off the period is of temporary influence or is non-cumulative in power. In these experiments, initial results were certainly followed by counter-

<sup>1</sup> The following summary gives sample results of the analyses of the period "composite" urines, which were carried out to check the daily results:

PERIOD.	TOTAL NITROGEN, IN GRAMS.			
	Composite urines.	Daily samples.	Differences.	
			Total.	Daily.
I.	104.30	<b>104.84</b>	0.54	0.045
II.	152.31	<b>152.68</b>	0.37	0.023
III.	89.48	<b>89.57</b>	0.09	0.009
IV.	<b>130.32</b>	128.70	1.62	0.125
V.	<b>220.48</b>	219.91	0.57	0.027
VI.	70.54	<b>71.23</b>	0.69	0.098
VII.	51.16	<b>51.69</b>	0.57	0.142

A year afterward, several of these samples were again analyzed, and found to have the same nitrogen-content. They had been preserved with powdered thymol. See MEAD and GIES, *loc. cit.*, p. 107, for a similar observation.



TABLE VI.

SUMMARY OF NITROGEN TOTALS FOR DAYS IMMEDIATELY PRECEDING AND FOLLOWING THE OPENING OF EACH PERIOD.

PERIODS.				NITROGEN. <sup>1</sup>				
No.	Days.	Conditions.	Fractions— numbers of days.		Total.		Balance a. <sup>2</sup>	
			Begin- ning.	End.	In- gested.	Ex- creted.	Total.	Daily average
I.	1-12	Normal . . . . .	....	8-12	grams 51.36	grams 50.15	grams +1.21	grams +0.25
II.	13-28	Anæsthesia, operation, and first hemorrhage (2.93 %) . . . . .	13-17	....	51.36	56.39	-5.03	-1.01
			....	24-28	51.36	52.17	-0.81	-0.16
III.	29-38	Anæsthesia alone . .	29-32	....	41.08	37.98	+3.10	+0.78
			....	35-38	41.08	40.45	+0.63	+0.16
IV.	39-51	Anæsthesia and opera- tion combined . . .	39-43	....	51.36	54.39	-3.03	-0.61
			....	48-51	41.08	41.65	-0.57	-0.14
V.	52-72	Second hemorrhage (3.22 %) . . . . .	52-58	....	71.90	85.50	-13.60	-1.94
			....	68-72	51.36	52.12	-0.76	-0.15
VI.	73-79	Third hemorrhage (3.51 %) . . . . .	73-75	....	30.81	35.01	-4.20	-1.40
			....	76-79	41.08	42.14	-1.06	-0.27
VII.	80-83	Fourth hemorrhage (3.26 %) . . . . .	Entire period.		41.08	55.25	14.17	-3.54

<sup>1</sup> The horizontal lines under nitrogen divide the figures in such a manner as to bring the results at the beginning of a period in immediate contrast with the similar results at the end of the previous period. The pairs of figures separated in this way in each column show at a glance the effects **before** and **after** the introduction of the new condition.

<sup>2</sup> See Table V.

balancing effects and were buried in the period totals. The columns of "period averages to date" show this very clearly. The effects of the hemorrhages, in the earlier periods especially, soon wore off, and the dog returned to nitrogenous equilibrium, although the period totals do not indicate it. These facts are shown conspicuously in Table VI.

The observed increase in nitrogen output was registered solely in the urinary constituents. Hemorrhage seemed to have no appreciable effect on the elimination of nitrogen in other excreta. The daily quantities of nitrogen in the faeces and hair were very uniform, as the figures in Table V will show. We found this to be the case, also, in the fractional periods indicated in Table VI.

In Table V the nitrogen of the cage-washings is included with that for urine, for the reason that practically all of this nitrogen came from urine in the first place. That the figures for nitrogen in the cage-washings do not affect our deductions in any way is evident from the following summary of average daily quantities thus recovered :

Periods . . . . .	I.	II.	III.	IV.	V.	VI.	VII.
Nitrogen, in grams .	0.153	0.150	0.197	0.195	0.164	0.140	0.145

The increased quantities of nitrogen in the washings of the third and fourth periods seem to have been due merely to the fact that urination was then more frequent, and, therefore, a larger amount of urine was each day mechanically held (and dried) at the bottom of the cage.

The catabolic losses of nitrogen after hemorrhage should be judged, also, in connection with the losses of nitrogenous matter from the blood. In Table V we have indicated the total as well as average daily loss of nitrogen from both sources, blood and excreta — "balance b." In considering the losses of nitrogen after the anæsthesia-operation period, for example, it must not be forgotten that with no removal of blood the effect in the direction of increased nitrogenous catabolism was relatively less than in the periods during which much blood was lost. The same is true in connection with phosphorus and sulphur compounds, and other metabolic products.

The ratio of nitrogen content, in grams, to urinary volume, in c.c., was 1 to 56, in the normal period. In the anæsthesia period (III), the ratio was 1 to 60. In the anæsthesia-operation period (IV), it was 1 to 55. In the hemorrhage periods it varied between 1 to 50, after the first, and 1 to 40 after the fourth loss of blood.

A regularly occurring feature in connection with nitrogenous equilibrium was the fact that although the food (uniform throughout) was apparently just right at the beginning of the experiment for the maintenance of metabolic balance, it was never found to be too great for the prompt restoration of nitrogenous equilibrium. It may have been temporarily in excess of the immediate needs of the animal, however, after the greatest losses following hemorrhage. The previous fact would indicate, perhaps, that the original equilibrium was established on a relatively low plane. Nitrogenous equilibrium was repeatedly re-established by the same amount of food in a dog gradually losing weight. Regenerative utilization of some of the nitrogenous matter doubtless accounts, in part at least, for the fact.<sup>1</sup>

*Chloride in the urine.* — The lowered nitrogen-content of the urine during the earlier part of the anæsthesia period (III), was a surprise because of the fact that the specific gravity remained about the same as previously, even though the volume was much increased. Repeated nitrogen determinations confirmed the accuracy of our original results. We were inclined to believe, at first, that the compensating influence might be due to increased excretion of phosphate, but the very small amounts of phosphorus-containing compounds in the urine could not have accounted for it. Determinations of chlorine, however, indicated the cause of the sameness of specific gravity, in spite of lessened elimination of urea and increased volume. The results summarized in Table VII show that chlorides were increased in the urine after ether anæsthesia. This increase was far in excess of any chloride that may have originated from the small amount of chloroform administered at the beginning of anæsthesia.

On adding to water, in a sample urinary volume (654 c.c.), an equivalent amount of chloride in the form of NaCl (7.2 grams), the specific gravity of the solution became 1009. On adding to this the twenty-ninth day's portion of nitrogenous matter in the form of urea (18.4 grams), the specific gravity rose to 1015. The specific gravity of the urine for that day was 1018, leaving 3 points unaccounted for,<sup>2</sup> and made up by the other constituents.

The increase in the specific gravity of the urine after hemorrhage in the other periods was doubtless due not only to diminished

<sup>1</sup> See the results of the third metabolism experiment.

<sup>2</sup> There are obviously several minor errors in such calculations, though they are approximately correct. See LONG: *Journal of the American Chemical Society*, 1903, xxv, p. 872.

excretion of water, but also to an increased output of chloride through the influence of the *anæsthetic*, and of urea as a result of the loss of blood.<sup>1</sup>

TABLE VII.  
URINARY CHLORINE BEFORE AND AFTER ETHER-ANÆSTHESIA.

PERIOD.		URINE.				PERIOD.		URINE.			
No.	Days.	Vol.	Sp. gr.	Cl.	N.	No.	Days.	Vol.	Sp. gr.	Cl.	N.
		c.c.		grams	grams			c.c.		grams	grams
II.	24	498	1019	1.82	9.41	III.	29	654	1018	4.37	8.59
	25	463	1021	1.71	9.28		30	495	1021	2.85	8.56
	26	444	1019	1.65	7.56		31	467	1019	2.73	8.80
	27	576	1019	1.87	10.33		32	388	1021	2.39	8.22
	28	615	1020	1.69	11.04		....	....	....	....	....
Average		519	....	1.75	9.52	Average		501	....	3.09	8.54

TABLE VIII.  
SUMMARY OF ANALYTIC DATA FOR TOTAL SULPHUR AND PHOSPHORUS METABOLISM.

PERIODS.		SULPHUR.				PHOSPHORUS.			
No.	Conditions.	Total.		Balance a. <sup>1</sup>		Total.		Balance a. <sup>1</sup>	
		Ingested.	Excreted.	Total.	Daily average.	Ingested.	Excreted.	Total.	Daily average.
		grams	grams	grams	grams	grams	grams	grams	grams
I.	Normal . . . . .	9.66	8.05	+1.61	+0.135	29.47	28.92	+0.55	+0.045
II.	Anæsthes., operation, and 1st hemorrhage (2.93%)	12.88	13.27	-0.39	-0.023	39.30	37.36	+1.94	+0.123
III.	Anæsthesia alone . . . .	8.44	7.39	+1.05	+0.105	24.57	24.42	+0.15	+0.015
IV.	Anæsthesia and operation combined . . . . .	11.47	11.65	-0.18	-0.014	31.94	29.84	+2.10	+0.163
V.	2d hemorrhage (3.22%) . .	16.83	20.64	-3.81	-0.180	51.29	50.86	+0.43	+0.022
VI.	3d hemorrhage (3.51%) . .	4.99	6.59	-1.60	-0.229	16.98	16.53	+0.45	+0.065
VII.	4th hemorrhage (3.26%) . .	2.85	4.24	-1.39	-0.348	9.70	10.43	-0.73	-0.180
<sup>1</sup> See Table V.									

<sup>1</sup> See pages 196, 203. Hemorrhage itself causes *diminished* elimination of chloride in the urine. KAST: Zeitschrift für physiologische Chemie, 1888, xii, 271.

*Sulphur catabolism.*—Our results for sulphur metabolism run parallel with those for nitrogen elimination, as the data in Tables VIII and IX indicate.

Our figures in this connection give the results of analysis of the various excreta for whole periods. Daily analyses, such as were made for nitrogen, would probably have shown that the greatest increase in the catabolism of sulphur compounds occurred during the days immediately following the hemorrhages. There was doubtless a return to the normal excretion, toward the ends of the periods, as in the case of nitrogenous catabolism.<sup>1</sup>

*Phosphorus catabolism.*—Tables VIII and IX also give our period data in connection with phosphorus-exchange. The tendency in the metabolism of phosphorus compounds was mainly the reverse of that shown by the nitrogenous and the sulphur-containing products, although the results are not very decided one way or the other. The phosphorus balance was on the side of retention. The excess of ingested over excreted phosphorus was least in the anæsthesia period (III), and greatest in the anæsthesia-operation period (IV). Although the results were not very striking, operation and hemorrhage, in most instances, seemed to cause slight retention of phosphorus; etherization favored increased elimination.<sup>2</sup> On the other hand, ether anæsthesia decreased the elimination of nitrogen and sulphur, whereas operation and hemorrhage increased the quantities of these elements in the excreta. We are unable to explain the variation from the usual order after the fourth hemorrhage.

As in the case of nitrogenous catabolism throughout this experiment, the differences in output of sulphur and phosphorus compounds were registered in the urine, not in the other excreta. This fact may be deduced from the data in Table IX.

*Total solids in the urine.*—In Table X we give the average daily quantities of total solids<sup>3</sup> in the urines passed during the first three days of each period. The catabolic results indicated by these figures are relatively the same as those of the data for nitrogen and sulphur. After the first hemorrhage, the elimination of total solids rose above

<sup>1</sup> See Table VI.

<sup>2</sup> In spite of the large quantities of phosphate in the food and fæces, we are confident our analytic data in this connection are without material error.

<sup>3</sup> Total solids were calculated from the figures for specific gravity. We used LONG's factor, 0.234 (at 20° C. referred to water at 4° C.). See LONG: *Journal of the American Chemical Society*, 1903, xxv, p. 262.

TABLE IX.  
SUMMARY OF ANALYTIC DATA FOR SULPHUR IN THE EXCRETIONS.

EXCRETIONS.	PERIODS.						
	I. 12 days.	II. 16 days.	III. 10 days.	IV. 13 days.	V. 21 days.	VI. 7 days.	VII. 4 days.
TOTAL SULPHUR OF EXCRETIONS.							
Urine and cage-washings . . . .	grams 5.509	grams 9.489	grams 5.317	grams 8.702	grams 15.616	grams 5.018	grams 3.277
Fæces . . . . .	2.244	3.340	1.871	2.701	4.618	1.450	0.890
Hair . . . . .	0.294	0.439	0.201	0.247	0.410	0.126	0.068
AVERAGE DAILY SULPHUR OF EXCRETIONS.							
Urine and cage-washings . . . .	0.459	0.592	0.532	0.669	0.743	0.717	0.819
Fæces . . . . .	0.187	0.209	0.187	0.208	0.219	0.207	0.225
Hair . . . . .	0.024	0.027	0.020	0.019	0.019	0.018	0.017
SULPHUR BALANCES.							
Total period balance <i>a</i> <sup>1</sup> . . . .	+1.613	-0.388	+1.046	-0.184	-3.812	-1.603	-1.383
Blood . . . . .	....	-0.680	....	....	-0.680	-0.680	-0.650
Total balance <i>b</i> <sup>1</sup> . . . .	....	-1.068	....	....	-4.492	-2.283	-2.033
Average daily balance <i>b</i> <sup>1</sup> . . . .	....	-0.067	....	....	-0.214	-0.326	-0.508
<sup>1</sup> See Tables V and VIII.							

the normal. In the anæsthesia and anæsthesia-operation periods (III and IV), total solids were also somewhat above normal in quantity, but below the amount excreted after the first hemorrhage. After the second hemorrhage (Period V), the increased elimination of solids was greater than after the first hemorrhage. After the third loss of blood (Period VI.), the solids of the urine, while still above

TABLE IX. — *continued.*  
SUMMARY OF ANALYTIC DATA FOR PHOSPHORUS IN THE EXCRETIONS.

EXCRETIONS.	PERIODS.						
	I. 12 days.	II. 16 days.	III. 10 days.	IV. 13 days.	V. 21 days.	VI. 7 days.	VII. 4 days.
TOTAL PHOSPHORUS OF EXCRETIONS.							
Urine and cage-washings . . . .	grams 5.759	grams 8.554	grams 4.949	grams 6.070	grams 10.983	grams 3.368	grams 2.409
Fæces . . . . .	22.300	28.670	19.400	23.680	39.720	13.110	7.995
Hair . . . . .	0.862 <sup>1</sup>	0.132	0.069	0.088	0.155	0.051	0.025
AVERAGE DAILY PHOSPHORUS OF EXCRETIONS.							
Urine and cage-washings . . . .	0.479	0.535	0.495	0.467	0.523	0.481	0.602
Fæces . . . . .	1.860	1.790	1.940	1.820	1.890	1.873	1.998
Hair . . . . .	0.072 <sup>1</sup>	0.008	0.007	0.007	0.007	0.007	0.006
PHOSPHORUS BALANCES.							
Total period balance a <sup>2</sup> . . . .	+0.551	+1.940	+0.147	+2.103	+0.429	+0.453	-0.725
Blood . . . . .	....	-0.220	....	....	-0.200	-0.200	-0.170
Total balance b <sup>2</sup> . . . .	....	+1.720	....	....	+0.229	+0.253	-0.895
Average daily balance b <sup>2</sup> . . . .	....	+0.107	....	....	+0.011	+0.036	-0.227
<sup>1</sup> High because of the presence of particles of fæces. In the succeeding periods all fæcal matter was separated from the hair before it was analyzed. See page 178. <sup>2</sup> See Tables V and VIII.							

the quantity eliminated after the first hemorrhage, were somewhat less in amount than after the second bleeding. The rise in amount of excreted solids was especially great after the fourth hemorrhage (Period VII), reaching the maximum of our records, just as was the case with nitrogen and sulphur elimination at the same time.

*Glycosuria.* — The facts in this connection in the third experiment, referred to on page 227, were subsequently verified in the urines of this experiment, also.

TABLE X.  
TOTAL SOLIDS IN THE URINE.

Conditions.	PERIODS.						
	I. Normal.	II. Anaesthesia, operation, and 1st hemorrhage.	III. Anaesthesia alone.	IV. Anaesthesia and operation combined.	V. 2d hemorrhage.	VI. 3d hemorrhage.	VII. 4th hemorrhage.
Total solids, in grams, per day	21.6	25.4	24.1 <sup>1</sup>	24.3 <sup>1</sup>	28.0	26.0	33.0

<sup>1</sup> Largely chloride. See Table VII.

TABLE XI.  
QUANTITIES OF FÆCES AND CAST-OFF HAIR FOR EACH PERIOD.

	PERIODS.						
	I. 12 days.	II. 16 days	III. 10 days.	IV. 13 days.	V. 21 days.	VI. 7 days.	VII. 4 days.
FÆCES.							
Total weight—	grams	grams	grams	grams	grams	grams	grams
Fresh . . .	400.9	524.4	359.3	455.4	768.8	308.0	164.0
Dry . . .	224.4	280.5	194.3	237.5	417.6	130.9	75.5
Av. daily weight—							
Fresh . . .	33.4	32.8	35.9	35.0	36.6	44.0	41.0
Dry . . .	18.7	17.5	19.4	18.3	19.9	18.7	18.9
CAST-OFF HAIR.							
Total weight . .	22.9	24.6	12.9	14.8	25.3	7.6	4.1
Av. daily weight .	1.9	1.5	1.3	1.1	1.2	1.1	1.0



*Fæces and cast-off hair.*—There seems to have been no particular effect on the amount or consistency of the fæces (page 188). Neither was there any observable influence on the quantity or chemical quality of the cast-off hair. See Table XI.

*Intestinal putrefaction.*—That there was little effect on intestinal putrefaction was shown by the uniformity not only in appearance of the fæces, but also in the urinary indican reaction.<sup>1</sup>

*Blood.*—Chemical analysis of the blood indicated that after the first hemorrhage, and during the two succeeding periods, the total proteid content of the blood increased, but that thereafter it decreased with each succeeding hemorrhage. These facts harmonize with the catabolic results for nitrogen and sulphur already noted. Although the total quantity of nitrogenous matter in the blood decreased, the phosphorus content remained practically the same. The compensating factor in this connection was doubtless increasing leucocytosis after each hemorrhage with augmented production of nucleoproteid. Increase of the latter substance in sufficient amount to keep the small phosphorus figure constant would not materially affect the results for nitrogen and sulphur. The specific gravity of the blood fell with the decrease in proteid matter and with the growing watery appearance noted at the times of withdrawal. In spite of its dilution in the last hemorrhage, the blood showed a very marked clotting tendency, which was due, of course, to the increased content of fibrinogen and perhaps other coagulation factors. See supplementary data, Table II.

#### IV. SECOND METABOLISM EXPERIMENT.

**A. First part. Influence of hemorrhage on proteid catabolism.**—Our second metabolism experiment was divided into two main parts. The first part was carried out on the plan of the preceding experiment, for the purpose of checking our former results. The conditions of the second part were somewhat different, as our intention was to obtain special data on volume of urine and on body-weight (page 212).

The animal made use of in this experiment was a slender, short-haired dog, weighing about 12 kilos. The dog of the first experiment was more fleshy and somewhat stronger.

<sup>1</sup> In special experiments now in progress in this laboratory we find that the amounts of combined  $\text{SO}_4$  in the urine are not particularly affected after hemorrhage.

**Diet.** — The food was the same in kind each day, and like that given throughout the first experiment. Its quantitative characters were uniform. Its composition is given in Table XII.

TABLE XII.  
COMPOSITION OF THE DAILY DIET.<sup>1</sup>

Ingredients.	Hashed beef. <sup>2</sup>	Cracker meal.	Lard.	Bone ash.	Water.	Total.
Weight . . . . .	grams 200	grams 52	grams 15	grams 8	grams 375	grams 650
Nitrogen . . . . .	7.518	0.806	0.004	0.002	....	8.330
Sulphur . . . . .	0.576	0.069	0.004	0.005	....	0.654
Phosphorus . . . .	0.440	0.070	0.013	1.423	....	0.195

<sup>1</sup> In the second part of the experiment the diet was changed somewhat. See Table XIV.

<sup>2</sup> See fifth footnote, page 179. The percentage-composition of this beef is there recorded as that of the "fourth preparation."

**Preparatory period.** — A preparatory period of seven days on the above diet was sufficient to get the dog into approximate nitrogenous equilibrium. During this time the weight of the animal fluctuated between 12.36 kilos and 11.85 kilos. At 5 P.M., on June 1, 1903, the collection of analytic data was started.

**First period. Normal conditions. Maintenance of nitrogenous equilibrium. Days, 1-9; June 1-9, 1903.** — The animal was further accustomed to its environment.

**Second period. Combined effects of anæsthesia and operation (without ligature of the artery). Days, 10-18; June 10-18.** — In the preceding experiment we determined the influence of anæsthesia and operation combined (Period IV). The operation of the first hemorrhage period (II) was exactly duplicated, even to the ligaturing and severing of the corresponding branches of the femoral artery. In that experiment, also, the first hemorrhage *preceded* the anæsthesia controls.

In this experiment we determined the combined influence of anæsthesia and operation *before* hemorrhage. We varied this control, also, by making the "operation" only an incision, without disturbance of the circulation. We laid bare the right femoral artery, just above the point at which the saphenous branch left it, and loosened adjacent tissue without doing any damage to the nerve. The incision was purposely made a little longer and the wound somewhat deeper, than at any time in the previous experi-

ment, so as to bring out the full effect on catabolism of the associated healing process.

*Schedule of operations.* — 8.22 A.M., anæsthesia begun. 8.55, incision made; exposure continued until 9.30, when the wound was sewed up and the anæsthetic discontinued. 10.00, dog sat up. The dog seemed to be much weaker at this stage than the previous animal, and was apparently more susceptible to the influence of the ether.

**Third period. Combined effects of anæsthesia, operation, and rapid hemorrhage. Days, 19-23; June 19-23.** — The combined effect of the anæsthesia and incision of the previous period was a well-marked stimulation of nitrogenous catabolism (Tables XIII-XV). Even though the dog was considerably smaller than the first one, the relative increase in excreted nitrogen was somewhat greater than in any of the *controls* of the previous experiment. The incision was the largest and deepest of any made in our experiments, and healing was very rapid, but it is improbable that this increased output of nitrogen was due to the healing process alone. It is possible, of course, that the ether had an opposite effect to that shown in the anæsthesia control of the preceding experiment. The fact noted above, that this dog was more susceptible to the influence of the anæsthetic than the previous one, may account for the observed difference in metabolism. Again, it may be that the effect of ether before hemorrhage is different from that afterward (see Period IV; also pages 183, 218).

In this period we next proceeded to ascertain the added effects of hemorrhage, and the interruption of circulation associated with it. It had been our intention to use for this purpose the femoral artery which had been exposed in the preceding operation, but the healing process was so rapid that a new incision became necessary. We therefore exposed the femoral artery at the same point on the opposite leg (left), making the incision as short and superficial as possible. In the previous experiment we observed a cumulative effect on nitrogenous catabolism with each hemorrhage, but each loss of blood came from a larger artery, and was associated with a greater disturbance of local circulation. In this case we took the blood from the main trunk of the femoral artery to begin with, thus effecting as great disturbance of circulation at the start as was caused at any time in the previous experiment.

The blood was drawn rapidly. The immediate effect on the dog was very decided, as indicated by changes in pulse and respiration, and the hemorrhage was discontinued somewhat earlier than had been intended. That this pronounced effect was due in part to the rapidity of withdrawal seemed evident from the fact that the dog rallied very quickly. More blood might well have been drawn.

*Schedule of operations.* — 8.15 A.M., anæsthesia begun. 8.35, operation

started. 8.53, hemorrhage commenced. 8.58, hemorrhage concluded. 9.15, wound sewed up. 9.20, anaesthesia discontinued. 9.50, dog sat up. The animal was apparently no weaker at this point than in the previous period.

TABLE XIII.

DAILY RECORDS OF THE "FIRST PART" OF THE SECOND METABOLISM EXPERIMENT.

I. First period. Maintenance of nitrogenous equilibrium. June 1-9, 1903.								
DAY. No.	BODY- WEIGHT.	URINE.					FÆCES.	
		Volume.	Specific gravity.	Nitrogen.	Period av'ge to date.		Weight.	
					Volume.	Nitrogen.	Fresh.	Dry.
	kilos	c.c.		grams	c.c.	grams	grams	grams
1	11.85	390	1016	7.63	390	7.63	22.8	15.8
2	11.80	415	1018	6.99	403	7.31	....	....
3	11.82	388	1020	7.58	398	7.40	50.2	29.3
4	11.70	485	1020	9.65	419	7.96	....	....
5	11.76	344	1017	5.31	404	7.43	....	....
6	11.60	515	1018	9.63	423	7.80	40.3	30.8
7	11.57	402	1019	8.14	420	7.85	34.2	21.4
8	11.63	298	1018	5.52	405	7.56	40.0	23.5
9	11.68	452	1020	9.12	410	7.73	41.5	24.3
II. Second period. Combined effects of anaesthesia and operation (without ligature of artery). June 10-18.								
10	11.40	450	1019	8.56	450	8.56	54.8	31.8
11	11.30	560	1019	9.46	505	9.01	....	....
12	11.28	484	1018	9.33	498	9.12	22.5	17.9
13	11.24	432	1019	8.66	482	9.00	....	....
14	11.26	422	1016	6.31	470	8.46	57.5	28.5
15	11.10	544	1023	12.55	482	9.14	....	....
16	11.06	383	1018	7.11	468	8.85	46.2	28.8
17	11.03	404	1020	8.00	460	8.75	29.1	19.1
18	11.00	480	1018	9.13	462	8.79	....	....

TABLE XIII—continued.

**III Third period.** Combined effects of anaesthesia, operation, and hemorrhage (3.11%). June 19-23.

DAY. No.	BODY- WEIGHT.	URINE.					FÆCES.	
		Volume.	Specific gravity.	Nitrogen.	Period av'ge to date.		Weight.	
					Volume.	Nitrogen.	Fresh.	Dry.
	kilos	c.c.		grams	c.c.	grams	grams	grams
19	10.47	442	1020	8.96	442	8.96	37.6	26.5
20	10.63	323	1028	9.59	383	9.59	....	....
21	10.60	418	1019	8.51	394	8.51	37.5	28.3
22	10.58	445	1023	9.94	407	9.94	....	....
23	10.48	503	1021	10.34	426	10.34	41.0	19.8

**IV. Supplementary data. Periods I-III.** [See also Tables XIV-XVI.]

BLOOD AND EXCRETIONS.	PERIODS.		
	I. 9 days.	II. 9 days.	III. 5 days.
	grams	grams	grams
<i>A. Urine :</i>			
Total nitrogen :			
Daily fractions . . . . .	69.570	79.110	47.340
"Composite" samples <sup>1</sup> . . .	(69.920)	(78.820)	(46.820)
<i>B. Cage-Washings :</i>			
Nitrogen . . . . .	0.880	0.720	0.870
Sulphur . . . . .	0.079	0.084	0.042
Phosphorus . . . . .	0.072	0.079	0.045
<i>C. Blood :</i>			
Percentage content :			per cent
Nitrogen . . . . .	....	....	3.168
Sulphur . . . . .	....	....	0.156
Phosphorus . . . . .	....	....	0.048

<sup>1</sup> See footnote, page 198.

*Blood.*—The amount of blood withdrawn was 342.5 grams, a quantity equal to 3.11 per cent of the weight of the dog at the end of the eighteenth day.

Nitrogenous excretion in this period was about 80 per cent greater than in the previous one (Tables XIII-XV). This result occurred in spite of the fact that the wound was the smallest made at any time in our experiments, and healed very rapidly. At the same time we did not check, in the preceding period, the influence of ligature and the consequent dis-

turbance of circulation in the limb. The dog immediately used both limbs with equal freedom, however, so that this disturbance must have been relatively slight.

- B. Second part. Influence of increased diet on recovery from hemorrhage.**—The first part of this experiment was intended to check the analytic results of the preceding one. In this part of our work we determined, as a final control, the effect of anæsthesia alone. We also ascertained the influence, on body-weight after hemorrhage, of an increase of both the solids and the water of the food. Further, after the dog had had sufficient time to recover pretty thoroughly from the effects of the first loss of blood (first part, nineteenth day), we subjected him to another, and almost fatal hemorrhage (forty-fourth day). Lastly, a few days having been allowed for partial recovery, we determined the effects of a further loss of blood on the flow of urine directly from the ureters of the same animal (page 231). In this part of our experiment only a few analyses were made of excreta. These were confined to the urine, our previous results having shown that metabolic changes were registered solely in the urinary products (page 200).

**Fourth period. Effects of anæsthesia alone. Days, 24–31; June 24–July 1.**

—In the first half of this experiment we checked the influence of anæsthesia with incision *before* hemorrhage. At this stage, *after* hemorrhage, the effect of anæsthesia was determined *without incision*. The results were essentially the same as in the first experiment. The anæsthetic was administered on the twenty-fifth day, after the results for urine volume on the previous day showed that conditions were favorable for the manifestation of its influence.

*Schedule of operations.*—9.30 A. M., anæsthesia begun. Continued in the usual manner until 10.30. 10.50, dog sat up.

**Fifth period. Influence of increased content of water in the diet on recovery from the effects of hemorrhage. Days, 32–35; July 2–5.**—

The usual temporary diuretic effect of the ether was clearly shown in the preceding period (Table XIV), the average daily volume (427 c.c. for the previous period) falling from 575 c.c. for the first two days to 443 for the seven days. On the last three days of the anæsthesia period the volume of urine was so uniform in amount, and so far below the usual elimination, and the dog showed such a uniform daily gain in weight, that retention of water seemed to be indicated. It seemed probable that more water was needed by the animal. Accordingly the amount of water in the food was increased by one-half its volume.

**Sixth period. Influence of increased content of water and solids in the diet on recovery from the effects of hemorrhage. Days, 36–43; July 6–13.**—The retention of water was quite decided in the previous period (Table XIV).

On the day before hemorrhage (eighteenth) the weight of the dog was 11.0 kilos. On the opening day of this period the weight was 10.39 kilos. Other facts, also, indicated that the animal had not yet recovered from the effects of the hemorrhage.

In this period, therefore, both the volume of water and the weight of solids in the food were increased one-half over the amounts given in the first four periods.

**Seventh period. Effects of a second (slow) hemorrhage, with increased diet. Days, 44-49; July 14-19.** — The condition of the animal at the end of the forty-third day was such as to indicate that it had almost entirely, if not wholly recovered from the effects of the previous treatment (page 215). Moreover, the animal was in a condition of progressive anabolism, due to the increased amount of food and drink it was receiving. The dog obtained much more water than it needed, and instead of being in nitrogenous equilibrium, as it was just before the previous hemorrhage, it was now daily storing up water and nitrogenous matter, and gaining in weight.

The diet of this period was the same as that of the sixth. At this hemorrhage the blood was drawn slowly from the saphenous branch of the right femoral artery. It had been our intention to remove blood equal in amount to about 4 per cent of the weight of the dog, but when 3 per cent of his weight had been taken, the blood flowed so slowly, even though there was no clot in the tube, respiration was so much impaired, and heart action so weak, that it seemed necessary to discontinue. In fact, we thought we had removed too much blood. While suturing the wound, however, the dog rapidly recovered, but he was much weaker during the rest of the day than at any time heretofore. Recovery gradually became complete. The blood was thinner than normal, it seemed to be a little darker in color, the red cells were diminished in number, and the leucocytes were more conspicuous than before.

*Schedule of operations.* — 8.15, anæsthesia begun. 8.35, operation started. 8.55, blood-letting commenced. 9.10, hemorrhage stopped. 9.12, administration of ether discontinued. 9.15, wound stitched. 10.20, dog sat up.

*Blood.* — The amount of blood withdrawn was 372.5 grams, a quantity equal to 3.44 per cent of the weight of the dog at the end of the forty-third day, or 3.39 per cent of body-weight at the end of the eighteenth day.

This hemorrhage also resulted in a distinct, though only slight increase in nitrogenous catabolism (Table XIV). This increase was greater, however, than that observed after the preceding hemorrhage, although the wound was smaller, and only a branch of the femoral artery ligatured.

**Flow of urine from the ureters after hemorrhage.** — See page 231.

**Supplementary data.**—The following additional facts should be noted with the observations recorded above. (Compare with data on page 187.) The fluctuations in the temperature of the room were between 20°–28° C. Temperature was usually about 24°–26° C. The wounds were not bandaged. Healing was very rapid. No signs of edema appeared. There were no instances of accidental hemorrhage. Our remarks on page 187 regarding losses in the weight of the previous dog on the day of operation, as well as those pertaining to the reaction of his urine, apply with

TABLE XIV.

DAILY RECORDS OF THE "SECOND PART" OF THE SECOND METABOLISM EXPERIMENT.

I. Fourth period. Effects of anæsthesia alone. June 24–July 1, 1903. N of diet = 8.33 grams; H <sub>2</sub> O of diet = 375 c.c.							
DAY.	BODY-WEIGHT.	URINE.				FÆCES.	
No.		Volume.		Specific gravity.	Nitrogen.	Weight.	
		Daily.	Average to date.			Fresh.	Dry.
	kilos.	c.c.	c.c.		grams	grams	grams
24 <sup>1</sup>	10.44	432	..	1017	9.08	40.4	22.0
25 <sup>2</sup>	10.39	528	528	1015	9.48	10.1	5.9
26	10.24	622	575	1014	8.67	42.6	24.1
27	10.29	371	507	1019	8.44	..	..
28	10.31	431	488	1016	..	35.2	21.4
29	10.32	386	468	1018	..	40.2	26.2
30	10.34	387	454	1017	..	..	..
31	10.39	378	443	1015	..	35.2	26.1

II. Fifth period. Influence of increased content of water in the diet on recovery from the effects of hemorrhage (third period, first part). July 2–5. N of diet = 8.33 grams; H <sub>2</sub> O of diet = 563 c.c.							
32	10.35	631	631	1013	..	..	..
33	10.41	540	585	1012	..	35.8	27.0
34	10.39	582	584	1012	..	..	..
35	10.31	644	599	1011	..	35.7	22.5

<sup>1</sup> Intermediate day. See page 212. See also footnote 2, next page, where data for this day are included with those for the third period.

<sup>2</sup> The anæsthetic was administered on this day.



TABLE XIV. — *continued.*

**III. Sixth period.<sup>1</sup>** Influence of increased content of water and solids in the diet on recovery from the effects of hemorrhage (third period, first part). July 6-13. N of diet = 12.49 grams; H<sub>2</sub>O of diet = 563 c.c.

DAY. No.	BODY-WEIGHT.	URINE.			FÆCES.		
		Volume.		Specific gravity.	Nitrogen.	Weight.	
		Daily.	Average to date.			Fresh.	Dry.
	kilos	c.c.	c.c.		grams	grams	grams
36	10.52	535	535	1015	..	..	..
37	10.41	624	580	1016	..	52.1	29.0
38	10.62	559	573	1017	..	..	..
39	10.67	551	567	1017	10.50	78.7	43.7
40	10.87	464	546	1017	9.01	..	..
41	10.85	590	554	1017	9.87	54.1	30.0
42	10.80	688	573	1016	10.05	52.5	27.9
43	10.83	631	580	1015	9.84	52.7	29.2

**IV. Seventh period.<sup>2</sup>** Effects of a second hemorrhage (3.44 %), with increased diet. July 14-19. N of diet = 12.49 grams; H<sub>2</sub>O of diet = 563 c.c.

44	10.37	534	534	1016	9.63	69.2	36.0
45	10.61	626	580	1021	12.25	..	..
46	10.53	670	610	1018	11.91	54.8	30.7
47	10.60	640	617	1019	12.32	..	..
48	10.63	593	612	1017	10.18	62.9	32.8
49	10.76	558	603	1018	..	38.3	20.1

<sup>1</sup> The days of the sixth period were extremely warm, but the temperature of the room in which the animal was kept did not go above 28° C.

<sup>2</sup> Supplementary data for nitrogen-content of the urine:

	III.	IV.	VI.	VII.
Periods . . . . .	3 days	3 days	5 days	5 days
Total nitrogen . . . . .	27.80 gms.	26.59 gms.	49.27 gms.	56.29 gms.
Average daily output of N. . .	9.27 gms.	8.86 gms.	9.85 gms.	11.26 gms.

equal pertinence to the same matters in this experiment. The former observations on specific gravity and reaction of the urine, on the qualities of the blood and fæces, on inhibited secretion of saliva during anæsthesia,

on respiration, blood-pressure, heart-beat, etc., after hemorrhage, were all confirmed in this experiment.

**Analytic results.** — Our more important daily results are recorded in Table XIII ("First part") and Table XIV ("Second part"). See also Tables XV and XVI.

**Discussion of results.** *First part.* — The figures in Table XIII show the following facts, among others, in harmony with the observations previously recorded (see page 193). There was a steady fall in the weight of the dog on a uniform diet, — from 11.85 kilos to 10.48 kilos. The daily volume of urine fluctuated between 298 c.c. (eighth day) and 560 c.c. (eleventh day). On the day of anæsthesia (tenth day), the volume of urine was *more* than the previous daily average, with the *maximal* volume on the second day of the period, and a steady decline to the *minimal* point on the fifth day. After the hemorrhage, on the other hand, the volume of urine on the same day was *less* than the previous average, fell on the following day to the *minimal* point in the period, and then steadily rose to the *maximal* point, on the fifth day of the period. This opposite tendency of the two conditions was as strongly marked as previously (see page 194).

The catabolism of nitrogen and sulphur compounds was stimulated somewhat after anæsthesia-operation (Period II) but more so after the hemorrhage. Only a slight effect was manifested on the output of phosphorus, but this was in the direction of decreased elimination after anæsthesia-operation and still more so after hemorrhage (see Table XV). The remarks made on page 198, regarding the appearance of counter-balancing effects at the ends of the periods, apply to this experiment also. That the increased catabolic effects were shown almost entirely in urinary products is seen at a glance in Table XV, under "average daily amounts in excretions," where the differences between "Balance a" and "Balance b" are also shown (page 197).

The significance of other data for the entire experiment may be seen in Table XVI.

*Second part.* — The figures in Table XIV show that the effect of anæsthesia alone in the fourth period (after a hemorrhage) was essentially the same as under similar circumstances in the first experiment, — decreased nitrogen catabolism, increased elimination of water. The effect of anæsthesia alone was different from that after anæsthesia with incision (Period II). These facts suggest that the healing process in the latter instance was responsible for the differ-



TABLE XVI.  
QUANTITIES OF FLEECES AND CAST-OFF HAIR, PERIODS I-VII.

	PERIODS.						
	I. 9 days.	II. 9 days.	III. 5 days.	IV. 8 days.	V. + days.	VI. 8 days.	VII. 6 days.
FÆCES.							
	grams	grams	grams	grams	grams	grams	grams
Total weight:							
Fresh . . . . .	229.0	210.1	116.1	203.7	71.5	290.1	225.2
Dry . . . . .	145.1	126.1	74.6	125.7	49.5	159.8	119.6
Average daily weight:							
Fresh . . . . .	27.0	23.3	23.2	25.5	17.9	36.3	37.5
Dry . . . . .	16.1	14.0	14.9	15.7	12.4	20.0	19.9
CAST-OFF HAIR.							
Total weight . . . . .	8.1	8.9	4.5	9.9	7.5	18.5	11.0
Average daily weight . .	0.9	1.0	0.9	1.2	1.9	2.3	1.8

ence (page 183), or that the effect of ether after hemorrhage is different from that before (page 209). The second hemorrhage (Period VII) produced the usual results. Thus, for example, during the five days preceding the hemorrhage (Period VI) the average daily excretion of nitrogen in the urine was 9.85 grams. During the five days following hemorrhage the average urinary nitrogen was 11.26 grams.

Reference is made on page 226 to the influence on body-weight, etc., of the changed dietary conditions prevailing during the last three periods.

*Glycosuria.*—The facts in this connection in the third experiment, referred to on page 227, were subsequently verified in the urines of this experiment also.

## V. GENERAL DISCUSSION OF THE RESULTS OF THE FIRST TWO METABOLISM EXPERIMENTS.

Our results seem to indicate that the body contains more blood at all times normally than the organism usually needs, and that some of this excess may be lost without particular detriment.<sup>1</sup> That this excess is in the nature of a reserve supply is indicated after hemorrhage by the prompt regeneration of volume, corpuscles, and soluble

<sup>1</sup> "Luxus blood" of Maragliano.

constituents. During special periods, of hard labor and the like, however, the body probably needs all of the oxygen resources of the whole supply of blood.

Each of our first two metabolism experiments has given results indicating a relatively slight and only temporary increase in nitrogenous elimination after hemorrhage, even when large volumes of blood were withdrawn and when the losses of blood occurred at short intervals. The catabolic effects became cumulative with each successive hemorrhage. This result was observed in spite of the fact that in the particular dogs under observation the anæsthesia itself appeared to exert an opposite effect. Under the very carefully controlled conditions of these experiments the catabolic effects were less striking than those referred to by Bauer and Jürgensen.

It is hardly necessary to remind the reader that our results in this connection are to be regarded as the balance of effects produced by several antagonistic forces. Loss of blood, if considerable, at once affects the higher nerve centres by diminishing the supply of material available for their nutrition. Such a changed condition of these nerve centres naturally disturbs various important chemical and physical functions. Immediately after hemorrhage, the "blood-making organs," chiefly the bone-marrow, are stimulated to exceptional activity. Increased activity of these parts after loss of blood results in unusual production of their normal catabolic products. These waste products soon appear in the urine, just as in the case of other groups of cells when stimulated to perform special labor.<sup>1</sup> Old erythrocytes disintegrate more quickly at first, but new ones are rapidly produced until the normal number is restored. New hæmoglobin is made. Fluid from the tissue spaces, and leucocytes from the lymphatic tissues, rapidly enter the blood-vessels until the volume is again normal and the colorless cells far exceed those usually present. The thirst and increased appetite of the animal under such circumstances show how much the organism needs new material for constructive purposes. Circulating and organized protoplasmic substance has been lost or decomposed. The supply of each kind is gradually renewed. Anabolism and catabolism are apparently in-

<sup>1</sup> An example of this may be cited in the observations by RIAZANTSEFF, among others, that nitrogen-elimination in the urine is increased during "sham feeding" of dogs because of the increased secretory activity of the digestive glands resulting therefrom. See HAMMARSTEN: *Lehrbuch der physiologischen Chemie*, 1899, p. 587.

creased in some parts of the body, and decreased in others, in response to the sudden call for a new supply of normal constituents. Sugar and other constituents are present for a while in the blood above the normal proportions.<sup>1</sup> That metabolism is also unusual in other respects, after hemorrhage, is evidenced by the fact that substances like albumin and lactic acid appear in the urine.<sup>2</sup> In the anabolic tendencies to replace constructive material, cellular matter appears to be worked over and a slight increase of nitrogenous and other waste matter is caused. All these changes take place on a plane of diminished resources and a small absolute increase of any constituent in the urine after hemorrhage is, in fact, a relatively large increase under the conditions then prevailing.

The more rapid influx of lymph into the blood after hemorrhage may have had something to do with the catabolic effects we have noted. That such abstraction of fluid from the tissue spaces does occur after hemorrhage has been shown repeatedly in various studies of blood-regeneration.<sup>3</sup> Immediately after hemorrhage the currents of diffusion from the tissue spaces are increased in speed and volume, specific gravity of the blood falls, and waste products, such as ammonia compounds, creatin, purin bases, and the like, may for a time appear in the blood in larger *proportion* than under normal circumstances, and may be excreted in the urine as a result of such quantitative abnormality. This appears to be true, also, of even the water of the blood.

In this connection, also, we are reminded of other influences upon nitrogenous excretion of increased elimination of fluid from the tissue spaces. The results obtained by several observers<sup>4</sup> lead us to the conclusion that the immediate withdrawal of fluid from the tissues after hemorrhage, to replace the volume of the blood, serves in itself to stimulate somewhat proteid catabolism, besides "washing out"

<sup>1</sup> SCHENCK: Jahresbericht der Thier-chemie, 1894, xxiv, p. 152.

<sup>2</sup> ARAKI: Zeitschrift für physiologische Chemie, 1894, xix, p. 424.

<sup>3</sup> The increased flow of lymph into the blood after hemorrhage, as shown, for example, by the steady increase in percentage-content of water (MEYER and GIES: results not yet published) seems to take place largely by transudation, — at first at least. Other experiments in progress in this laboratory show that after hemorrhage the flow of *thoracic* lymph is *diminished*. See POSNER and GIES: Proceedings of the American Physiological Society, 1904. This journal, x, p. xxxi.

<sup>4</sup> LANDAUER: Jahresbericht der Thier-chemie, 1894, xxiv, pp. 531, 532. Also STRAUB, cited by SPECK: Ergebnisse der Physiologie zweiter Jahrgang (Bio-chemie), 1903, p. 26.

the catabolic products already in and about the cells. The feeding conditions of our experiments were, however, against a striking effect in this direction, for more than enough water to replace the losses was received shortly after hemorrhage.

We observed that after hemorrhage urinary volume was at first diminished, then increased, and that after a few days it fell to approximately the normal again. An examination of the various tables also shows that the increase in nitrogenous elimination accompanied the increase in volume of urine. The condition of the animal shortly after hemorrhage is similar in many respects to that after fasting. It is not improbable, therefore, that the quantity of water given *uniformly in each period* was, for a time after each hemorrhage, actually in excess of the amount the animal could at once make use of. This seems to be indicated by the steady rise in the figures for average daily volume of urine of each hemorrhage period, as body-weight and the plane of equilibrium fell. Consequently part of the observed cumulative increase in excreted nitrogenous compounds after each hemorrhage may have been due to the action of such relatively increasing excesses of water in the food.<sup>1</sup>

It is not improbable that during the first few hours after a hemorrhage there may be subnormal metabolism, followed by a greatly stimulated metabolism. The fall in blood-pressure and in temperature immediately after hemorrhage, and the rapid return to the normal, with even a higher temperature than usual, and a somewhat greater consumption of oxygen, facts observed by various investigators, tend to support such a view. That moderate hemorrhage has a stimulating influence has been asserted recently by Robin<sup>2</sup> and others, after many years of observation of the effects of blood-letting on man. But that the effects of even a "moderate" hemorrhage are *at first* somewhat depressant has also been frequently observed.

In all probability, as Jürgensen and others have stated, the increase of eliminated nitrogen is relatively much greater after hemorrhage in fasting animals than in well-nourished ones. In the latter each new supply of food aids the cells in the increased anabolic changes at once set up after the hemorrhage. In the former instance the cells themselves must furnish much of the new material needed to restore the blood and keep its composition constant, and after such en-

<sup>1</sup> VOIT: HERMANN'S Handbuch der Physiologie, 1881, Bd. vi, Th. i, p. 153.

<sup>2</sup> Cited by SMALL: Reference Handbook of the Medical Sciences, 1901, ii, p. 71.

forced and unusual intracellular modifications the waste of material is probably greater than when extracellular sources are chiefly drawn upon in the usual manner. It is probable, as we have already suggested, that immediately after hemorrhage the animal, even if previously well nourished, is in a condition similar to that of the fasting animal, and that, in spite of its immediate requirements, a large part of the proteid nitrogen of the food (if the dog had been previously in equilibrium) may be quickly eliminated before all the needs are supplied. In our experiments it is possible that the diet, which was just sufficient to maintain equilibrium before hemorrhage, was more than enough to do so just after the loss of blood, and that it remained excessive until equilibrium was established on a lower level of body-weight, etc. The steady increase in daily average volume of water eliminated by the kidneys lends support to such a conclusion. Under such conditions the temporary excesses of proteid in the diet would themselves stimulate catabolic processes.

The work of Bauer, Voit and Rauber, Pembrey and Gürber, and others, makes it probable that there was little, if any, interference with general oxidation after hemorrhage in these experiments, and that our results are not to be viewed from the standpoint of particularly abnormal internal respiration.<sup>1</sup> Stimulated heart-action and quickened lymph currents after moderate hemorrhage result in maintaining the normal gaseous exchange. We are unable to accept Fränkel's deduction that oxidation after moderate hemorrhage is necessarily greatly reduced for any great length of time, nor to attribute to such assumed reduction, as he does, the posthemorrhagic increase in proteid catabolic products in the urine. That total muscular power is lessened immediately after hemorrhage stands to reason, and has been shown by Jürgensen and others, but there is as much evidence of quickened gas metabolism in the *resting* animal, after hemorrhage, as the reverse—observations in harmony with the fact that oxidation occurs almost entirely in the tissues and only slightly in the blood itself.<sup>2</sup> That oxidation was at first lessened somewhat in the limb operated on was, of course, always the case, especially when the blood was drawn from the femoral artery itself and its main trunk permanently closed. This was shown in the im-

<sup>1</sup> SPECK: *Ergebnisse der Physiologie*, zweiter Jahrgang (Biochemie), 1903, p. 23.

<sup>2</sup> The slight fall in temperature immediately after hemorrhage is probably due to greater radiation rather than lessened production of heat.



mediate fall of temperature in the leg from which blood was withdrawn. But the normal temperature was soon restored, there was little interference with the use of the limb, and there is no reason to believe that the metabolic effects of this interruption of circulation were appreciable.

The sulphur-elimination ran parallel with the nitrogenous, as is usually the case when proteid catabolism is increased.<sup>1</sup> But hemorrhage seemed to cause a variable effect on the excretion of phosphorus compounds, mainly a decrease. We are inclined to attribute this decrease to the stimulated nuclear anabolism following loss of blood (increased number of leucocytes and production of nucleated red cells), with the consequent retention of phosphorus because of nucleoproteid formation.<sup>2</sup> Possibly in this special utilization of phosphorus in nuclear anabolism, nitrogen and sulphur radicles are broken away from the proteid elements transformed in the process.

It has been stated by several writers that acute anæmia interferes decidedly with digestion,<sup>3</sup> and Jürgensen quotes Ranke to the effect that the flow of bile is markedly diminished after loss of blood.<sup>4</sup> Manassein<sup>5</sup> stated that after hemorrhage the gastric glands secreted less acid than normally and also a less active juice. Recently it was observed by London and Sokolow<sup>6</sup> that loss of blood effects a decided inhibition of the total secretion of gastric juice. This decided effect was noted by them even when the ordinary stimuli were in operation, and for periods as long as six hours after the beginning of acute anæmia. The character and amount of the fæces, the apparent relish with which the food was taken after each hemorrhage, and the entire lack of vomiting indicate that in these experiments there was no appreciable effect on the process of digestion as a whole (pages 185, 188).

We have already noted the fact that the wounds connected with the hemorrhages were not bandaged. The dog was permitted to lick them. Although a small amount of proteid matter was carried

<sup>1</sup> SHERMAN and HAWK: This journal, 1900, iv, p. 25.

<sup>2</sup> In *fasting* dogs, LEPINE and FLAVARD observed increased elimination of  $\text{PO}_4$ . Cited by HAYEM: *Leçons sur les modifications du sang*, 1882, p. 328: JÜRGENSEN also noted an increase.

<sup>3</sup> FLINT: Text-book of physiology, 1882, p. 251.

<sup>4</sup> HEIDENHAIN: HERMANN'S Handbuch der Physiologie, 1883, Bd. v. Th. i, p. 263.

<sup>5</sup> MANASSEIN: Jahresbericht der Thier-chemie, 1872, ii, p. 217.

<sup>6</sup> LONDON and SOKOLOW: Centralblatt für Physiologie, 1903, xvii, p. 179.

in this way from the wound to the stomach, and there digested, no appreciable effect could have been exerted by such a trifling variation. The increase of eliminated nitrogen was always highest immediately after the operation. By the time the lips of the wound parted, and the dog could lap the little exudate then forming, the nitrogen balance was restored (page 188).

Bauer and Jürgensen both state that the surgical procedure in their experiments had no observable effects. Our own data indicate that the operation or the healing process or some associated factor connected with each hemorrhage had an appreciable, though slight, influence in increasing the output of catabolic products. The wound was always small and superficial,<sup>1</sup> healing rapidly, with only slight inflammation and scarcely any exudation. The attendant leucocytosis may have been a contributing catabolic as well as anabolic factor. But by the time the healing process might have been supposed to exert *special* influence on metabolism, the observed posthemorrhagic effects had practically worn off, and the dog was again in equilibrium. Effects caused by interference with local circulation (resulting from ligation of the blood-vessel) were not determined directly. Stasis naturally interferes to a certain extent with functional activity by reducing the nutrition of the cells, and because of the pressure of accumulated plasma. Edema was observed only once (page 187). Acute anæmia below the ligation would likewise interfere with nutritional activity. But at various times after some of our operations blood promptly appeared when slight incisions were made through the skin of the foot. The results of the last two or three hemorrhages in our first experiment, when the main trunk of the femoral artery was entered, might be attributed in part to greater local destruction of proteid matter, and increased absorption of nitrogenous material into the lymph and thence into the circulation. At the same time the results of our second experiment indicate that such an effect must have been inconsequential. The ease with which the animals always used their limbs, even when each femoral artery had been ligatured, suggests that, even if interfered with to a certain extent at first, metabolism must have been essentially the same in the limbs afterward, because of sufficient collateral circulation to maintain approximately normal conditions of oxidation, lymph flow, etc.

<sup>1</sup> The exception is referred to on page 208.

## VI. THIRD METABOLISM EXPERIMENT.—INFLUENCE OF HEMORRHAGE ON BODY-WEIGHT.

There are many statements in the literature of the subject of blood-letting to the effect that repeated losses of blood result in general fattening, with a consequent increase of body-weight. Fatty degeneration of the heart, capillary system, and various glands has been observed to occur in many individuals subjected to numerous hemorrhages. Bauer likens this effect to that following phosphorus poisoning. Various clinical reports agree with Van Swieten's references to a woman who gained 150 pounds in weight as a result of sixty bleedings in a few months, and Lister is repeatedly quoted as saying that in England calves were made so fat by frequent bleedings that the serum became like milk.<sup>1</sup>

In our first metabolism experiment we started with a dog that was in nitrogenous and weight equilibrium. In spite of the fact that the same amount and kind of food was fed each day throughout the entire experiment, the dog failed to regain the weight lost with each hemorrhage. Although the experiment continued for eighty-five days,<sup>2</sup> there was a fairly uniform loss of weight, from 16.8 to 12.6 kilos, with little or no recovery at any time.<sup>3</sup> This experiment seemed to make it evident that the increased weight noted by Talmatscheff and other previous investigators was not due to hemorrhage directly, but indicated that changed feeding conditions after the bleedings were perhaps largely if not wholly responsible for such observations.<sup>4</sup> After even a moderate hemorrhage, thirst is experienced and appetite is increased. In gratifying this special desire for food and drink, much more than enough to meet the real needs of the body would doubtless be taken by the subject in anæmia, with a con-

<sup>1</sup> Cited by JÜRGENSEN.

<sup>2</sup> We are unable to find any record of a longer experiment of this kind.

<sup>3</sup> This general result has again been obtained in experiments of a similar character now in progress in this laboratory.

<sup>4</sup> It may also be possible that hemorrhages of small amount (1 to 2 per cent) have a different effect from losses of large quantities (3 to 4 per cent), even on a constant diet. The results obtained by TALMATSCHIEFF and others harmonize with this view, although TALMATSCHIEFF's dogs were not brought into equilibrium to begin with, and it is therefore impossible to draw any very definite conclusions, in this connection, from his observations. TALMATSCHIEFF: HOPPE-SEYLER'S *medicinisch-chemische Untersuchungen*, 1866, p. 396.

sequent storing up of constructive material and a necessary gain in weight.

This deduction was tested in the second half of the second experiment. In the fifth period of that experiment it was found that a 50 per cent increase in the amount of water in the food resulted in the retention of only a small portion of the extra amount. The weight remained the same. But in the sixth period, with an increase, also, of 50 per cent of the solid matter in the food, there was still more retention of water, a storing up of solids, and a steady increase in weight,—effects which seemed to confirm the explanation offered by us for *much* of the usual posthemorrhagic gain in weight. These results in the second metabolism experiment were obtained rather late, however, in the period of recovery from hemorrhage. Immediately after loss of blood they might have been even more decided. We observed that the food was not as great in quantity as the dog desired it to be. If his appetite had been fully satisfied, the gain in weight might have been greater.

In the third metabolism experiment these matters were further tested as follows:

A healthy, well-nourished dog weighing 10 kilos was offered an excess of the mixed diet used in the previous experiments, and was allowed to take it in such amounts as his desires determined. The feeding occurred daily at 5 P. M. The weight of the dog was taken at 9 o'clock each morning. At intervals blood was drawn in moderate amount from the femoral artery. Two withdrawals were made from one artery and three from the other during the experiment. The operations were conducted under aseptic conditions, and the wounds healed rapidly with a minimum amount of inflammation. Ether was the anæsthetic used in the operations. The experiment continued for about six weeks. The essential facts in the record of results are indicated in Table XVII, on the following page.

The dog became quite stout during the latter part of the experiment, used his legs freely at all times, recovered quickly from each loss of blood, and suffered no apparent ill effects from the treatment. The animal's appetite was always especially strong after the hemorrhages, particularly after the last three.

The influence of full gratification of the appetite after hemorrhage seems to be very clear in this experiment. Immediately after the first hemorrhage there was the usual loss of weight by difference. But on a free diet, increased appetite soon sent the weight up again.

TABLE XVII.  
BODY-WEIGHT AFTER SUCCESSIVE HEMORRHAGES.

BODY-WEIGHT.		BLOOD WITHDRAWN.		
Day of record.	Kilos.	Day of record.	Grams.	Percentage of body-weight.
1	10.56 <sup>1</sup>	1	317	3.0
8	10.30 <sup>2</sup>	12	319	3.1
15	10.54	18	304	2.7
22	10.85	20	228	2.0
29	11.57	29	250	2.2
36	12.33	..	..	..
43	13.17	..	..	..

<sup>1</sup> During the preliminary period, on the same dietary conditions, the weight of the dog fluctuated between 10.6 and 10.1 kilos.

<sup>2</sup> The minimum weight was recorded on the fourth day, when it fell to 9.9 kilos.

Each successive hemorrhage showed a cumulative effect in this direction in spite of the losses of the blood itself. Although 1.2 kilos of blood was taken during the first twenty-eight days, the body-weight increased a kilo in the meantime. The gain in weight was even more rapid later, when blood-letting was discontinued.

*Pathological constituents* were looked for in the urines of this experiment.<sup>1</sup> The only observed abnormality was a *slight* transient glycosuria after the first two hemorrhages. This result was attributed to the anæsthetic. It led us to examine the urines of the previous experiments, which had been carefully preserved with powdered thymol.

On testing the urines of the first metabolism experiment, sugar was detected in the anæsthesia (III), anæsthesia-operation (IV), and second hemorrhage (V) periods but in no others. In the urines of the second metabolism experiment, sugar was found after anæsthesia-operation (II), and anæsthesia alone (IV), but not after anæsthesia and bleeding combined.<sup>2</sup> In the latter two experiments the glycosu-

<sup>1</sup> The urines were tested for the following substances, by the usual methods: sugar, albumin, proteoses, diacetic acid, acetone, bile pigment.

<sup>2</sup> This result was obtained by Mr. WHITE and verified by Dr. GIES, after Dr. HAWK retired from this department to begin his work at the University of Penn-

ria may have been due in part to the chloroform administered in the early stages of anæsthesia. Schenck, Araki, Rose, and others have observed an increased percentage-content of sugar in the blood after hemorrhage. This fact might lead us to expect a special elimination of sugar in the urine after bleeding, but Schenck found that at the time of hemorrhagic glycemia the urine was, nevertheless, free from sugar.<sup>1</sup> In later experiments, in which we obtained urine from the ureters of dogs, in morphia-atropin narcosis, sugar was absent, in any appreciable amount, both before and after hemorrhage.

#### VII. THE INFLUENCE OF HEMORRHAGE ON THE FLOW OF URINE.

In each of our metabolism experiments hemorrhage always caused a temporary *decrease* in the excretion of urine, to be followed by stimulation for a day or two. Anæsthesia and anæsthesia combined with the operative procedure, were invariably followed, within twenty-four hours, by a temporary *increase* in the flow of urine (see Tables II, III, IV).

The observed diuretic influence of the ether was unexpected, for statements have been repeatedly made that this anæsthetic inhibits the flow of urine. Thus, Lawson Tait observed that when ether anæsthesia became profound urine entirely stopped flowing from the ureters.<sup>2</sup> Kemp recently called attention to the contracting influence of ether on the renal arterioles of the dog, by which effect the flow of urine is much diminished, or may be stopped entirely.<sup>3</sup> Anæsthesia in all our experiments was *very light; just enough to keep the animal quiet*. Whatever inhibition of urine flow there may have been, was surely of short duration, and must have been followed, as our results show, by actual stimulation. The current statements to the effect that ether anæsthesia causes concentration of the blood are of interest in this connection.<sup>4</sup>

As has already been indicated, our original purpose had been to investigate the effects of ether itself, besides those of hemorrhage, but lack of time for our work in collaboration prevented (page 175). It was evident incidentally in all of the preceding experiments, however, that under the prevailing conditions of anæsthesia, ether stimulated a daily flow of urine. Hemorrhage, on the other hand, was

sylvania. Dr. HAWK there independently noted a similar glycosuria in dogs in his study of the effects of ether anæsthesia. See page 176, footnote.

<sup>1</sup> VON NOORDEN: *Lehrbuch der Pathologie des Stoffwechsels*, 1893, pp. 316, 337.

<sup>2</sup> Cited by PATTON: *Anæsthesia and Anæsthetics*, 1903, p. 101.

<sup>3</sup> Cited by CUSHNY: *Text-book of Pharmacology and Therapeutics*, 1901, p. 158.

<sup>4</sup> PATTON: *Loc. cit.*

immediately followed by decided inhibition of flow, in spite of the counter-influence of the associated anæsthesia (Table III). The subsequent stimulation of excretion in all of the hemorrhage periods of our metabolism experiments may have been dependent on the increased percentage-content of water in the blood.<sup>1</sup>

In a few special experiments we attempted to ascertain more directly the facts connected with urine flow after hemorrhage. These experiments were conducted by the usual methods. Well-nourished dogs were subjected to light morphia-atropin narcosis,<sup>2</sup> and cannulas placed in the ureters. Care was taken to prevent undue pressure on or occlusion of the latter. The animal was kept under suitable covering, and temperature was maintained as well as possible, with the aid of a hot water bag.<sup>3</sup> After preliminary observations of the flow of urine under these conditions, blood was drawn from a large artery, as in previous experiments, and the subsequent flow of urine again observed. The data of two of our experiments will suffice here to show the essential points observed in this connection.

**First experiment. Effects of two hemorrhages.** — A dog weighing 26 kilos was used. The animal had been well fed previously. A heavy meal was eaten late the night before. Narcotics were injected at 9.20 A. M. Operation was begun at 10.30. Ether was used occasionally, when necessary. Cannulas were placed in the ureters, and into the left femoral artery, and unobstructed flow of urine was observed at 11.10 A. M. The following data are quoted directly from our records:

**Preliminary period:**

QUANTITY OF URINE COLLECTED.

TIME.	FRACTIONS.		TOTAL.	
	Right kidney.	Left kidney.	20 minutes.	1 hour.
a.m.	c.c.	c.c.	c.c.	c.c.
11.12-11.32	2.75	2.60	5.35	..
11.32-11.52	2.45	2.60	5.05	..
11.52-12.12	2.80	2.80	5.60	..
Total per hour	8.00	8.00	..	16.00

<sup>1</sup> MEYER and GIES find, in experiments now in progress in this laboratory, that even during a continuous hemorrhage the percentage-content of water in the blood steadily rises to the end. See page 193.

<sup>2</sup> A concentrated aqueous solution of morphine sulphate (6 mgms. per kilo) and atropin sulphate (0.6 mgm. per kilo) was injected subcutaneously.

<sup>3</sup> The animal did not come in contact with the bag. The latter merely kept warm the air under the blanket covering the dog.

*First hemorrhage.* — 12.12, first hemorrhage was begun; continued until 12.19. Amount of blood withdrawn was 829.5 grams = 3.14 per cent of body-weight. 12.13, flow of urine from left kidney decreasing rapidly. 12.19, same true of flow from right kidney. 12.20, *flow of urine stopped entirely*. 12.40, first sign of renewed flow, from each kidney simultaneously.

## QUANTITY OF URINE COLLECTED.

TIME.	FRACTIONS.		TOTAL.	
	Right kidney.	Left kidney.	20 minutes.	1 hour.
p. m.	c.c.	c.c.	c.c.	c.c.
12.20-12.40	0.0	0.0	0.0	..
12.40-1.00	1.70	1.70	3.40	..
1.00-1.20	2.30	2.20	4.50	..
Total (40 min.)	4.00	3.90	..	7.90
1.20-1.40	2.45	2.40	4.85	..
1.40-2.00	2.35	2.25	4.60	..
2.00-2.20	2.75	2.70	5.45	..
Total per hour	7.55	7.35	..	14.90
2.20-2.40	2.80	2.70	5.50	..
2.40-3.00	2.70	2.80	5.50	..
3.00-3.20	3.00	3.00	6.00	..
Total per hour	8.50	8.50	..	17.00

*Second hemorrhage.* — At 3.20, second hemorrhage was begun; continued until 3.26. Amount of blood withdrawn was 403 grams = 1.55 per cent of body-weight. [Total amount of blood in both hemorrhages, 1232.5 grams = 4.69 per cent of body-weight.] 3.27, flow of urine from each kidney decreasing; *suspended entirely at 3.29*. 4.15, excretion from right kidney beginning; at 4.20 from the left.

8.40, flow of urine has continued very slowly since 4.15 — 1.5 c.c. from the right kidney, 1.7 c.c. from the left. The animal did not recover from the shock of the second hemorrhage. At 9.00 the urine had ceased flowing. The animal died at 11.15. Heart-action, respiration, and blood-pressure, accorded with the usual phenomena under similar circumstances.



In this experiment it was evident that a hemorrhage of 3 per cent of body-weight stopped the flow of urine immediately, but the interference lasted for only about a half hour. In three hours, however, the flow gradually returned to the normal quantity per hour and began to exceed it. A second hemorrhage proved fatal, but at first it also was followed by complete inhibition of the flow of urine and then by gradual resumption as before. The results in the latter connection indicate that if the first hemorrhage had been greater, and yet not fatal, the initial inhibitory effects would have been as marked as they were in our metabolism experiments. It is quite probable, also, that the subsequent stimulating effects would have appeared as late as they did in those experiments.<sup>1</sup> See page 194.

**Second experiment. Effects of two hemorrhages and subsequent return of each portion of blood (defibrinated).—**The animal taken for this experiment was the one used in the second metabolism experiment.<sup>2</sup> The dog weighed 10.85 kilos. He had been well fed during the previous experiment in which he had been used, and at 8 A. M., on the day of this experiment, had eaten a hearty meal. Narcotics were injected at 11.15 A. M. At 12.15 the operations were begun; completed at 12.35. Ether was used as occasion required. Cannulas were put into the ureters and into a carotid and jugular. Blood was drawn slowly from the carotid. Later, after having been defibrinated and filtered, the blood was returned, at 38° C., by way of the jugular. Further details are quoted directly from our summaries.

**Preliminary period :**

QUANTITY OF URINE COLLECTED.

TIME.	FRACTIONS.		TOTAL.	
	Right kidney.	Left kidney.	15 minutes.	5 minutes.
p. m.	c.c.	c.c.	c.c.	c.c.
12.40-1 10	7.4	7.9	7.7	2.5
1.10-1.40	7.0	7.4	7.2	2.4
Total per hour	14.4	15.3	..	..
1.40-2.25	10.9	10.5	7.1	2.4

<sup>1</sup> The different effects of the narcotics employed must, of course, also be taken into account.

<sup>2</sup> That experiment was concluded on July 19, 1903. This experiment was carried out two days later.

*First hemorrhage.* — At 2.25 first hemorrhage was started ; concluded at 2.30. Amount of blood withdrawn was 387 grams = 3.57 per cent of body-weight. 2.26, *flow of urine stopped entirely*. Breathing slow and shallow. Heart-beat very rapid. Pulse almost imperceptible. Blood-pressure very low. 2.28, hemorrhage nearly fatal. Occasional gasps. Respiration stimulated mechanically until 2.50, when improvement was noticed. 2.55, respiration and heart-beat approximately normal. 3.10, *urine suddenly began to flow from each cannula*.

## QUANTITY OF URINE COLLECTED.

TIME.	FRACTIONS.		TOTAL.	
	Right kidney.	Left kidney.	15 minutes.	5 minutes.
p. m.	c.c.	c.c.	c.c.	c.c.
3.10-3.25	3.0	1.5	4.5	1.5
3.25-3.40	3.1	2.0	5.1	1.7
Total per $\frac{1}{2}$ -hour	6.1	3.5	..	..

*First return of defibrinated blood.* — At 3.40 began injection of blood into the jugular ; concluded at 3.54, when all had been returned. No effect on flow of urine was evident until about 200 c.c. had been injected, — at 3.43, *when the flow from each cannula increased*. Respiration gradually became deeper and slower, the pulse firmer and less rapid. After 3.53 the urine leaving the cannulas contained an increasing though only small amount of hæmoglobin, but no corpuscles [hæmoglobin had probably been liberated into the serum in the defibrination process].

## QUANTITY OF URINE COLLECTED.

TIME.	FRACTIONS.		TOTAL.	
	Right kidney.	Left kidney.	15 minutes.	5 minutes.
p. m.	c.c.	c.c.	c.c.	c.c.
3.40-3.45	3.0	2.5	..	5.5
3.45-3.50	3.0	2.5	..	5.5
3.50-3.55	3.3	3.9	18.2	7.2
3.55-4.00	3.1	3.0	..	6.1
4.00-4.05	2.9	2.5	..	5.4
4.05-4.10	3.0	3.0	17.5	6.0
Total per $\frac{1}{2}$ -hour	18.3	17.4	..	..

*Second hemorrhage.* — At 4.11 the second hemorrhage was started; concluded at 4.21. Amount of blood taken was 330 grams = 3.04 per cent of body-weight. 4.12, *flow of urine stopped entirely*. Respiration and heart-beat not seriously affected this time. No flow of urine up to 4.41.

*Second return of defibrinated blood.* — At 4.42 injection of blood was begun. No effect on urine flow until 4.48, when 200 c.c. of blood had been injected. At 4.48 *urine began to flow rapidly*. No more was injected.

At 5.20 the animal was chloroformed and the experiment discontinued.

QUANTITY OF URINE COLLECTED.

TIME.	FRACTIONS.		TOTAL.	
	Right kidney.	Left kidney.	15 minutes.	5 minutes.
p. m.	c.c.	c.c.	c.c.	c.c.
4.48-5.03	11.5	9.5	21.0	7.0
5.03-5.18	7.5	6.5	14.0	4.7
Total per $\frac{1}{2}$ -hour.	19.0	16.0	..	..

In this experiment the results were similar to those of the preceding one. Hemorrhage of 3.5 per cent of body-weight resulted in immediate cessation of the flow of urine. For three-quarters of an hour urine failed to appear. In the previous experiment the first inhibition period was shorter, evidently because the amount of blood withdrawn was less and the hemorrhage was not so nearly fatal. After the second hemorrhage in the previous experiment, however, this interval was about the same. On returning about one-half of the blood, urine at once began to flow, exceeding in a few minutes the normal amount. Repetition of the process gave the same results.

Immediately after hemorrhage, blood-pressure sinks, and there are other symptoms of shock. After a three to four per cent loss of body-weight in blood there is obviously sufficient fall in blood-pressure to entirely prevent urine formation for a period of varying length. Vasomotor influences, together with the increasing volume of inflowing lymph, doubtless operate to restore the usual pressure and normal conditions in a relatively short time, as may be done at once by returning the blood itself. The continued influx of lymph

after hemorrhage not only raises blood-pressure, but also increases the water-content of the remaining blood. This increasing content of water in the blood, together with the absolute increase in volume after the return of the blood, doubtless accounted for the increased output of water in the urine after the initial posthemorrhagic interruption of flow had ceased (see page 194).

These results confirm the early observations of Goll<sup>1</sup> and the subsequent deductions of Bauer.

### VIII. SUMMARY OF GENERAL CONCLUSIONS.

*A.* External hemorrhage, equal to from 3 to 3.5 per cent of the body-weight, of dogs, was observed to cause the following more important effects:

In well-nourished animals, in weight and nitrogen equilibrium, and fed continuously on a diet of constant composition, there was a temporarily increased output of nitrogenous and sulphur-containing products in the urine, and a variable effect on the elimination of phosphorized substances, though mainly a decreased excretion of the latter. Total solids in the urine were increased with the nitrogen and sulphur catabolism. These effects were relatively slight after one bleeding of moderate amount, but became more marked and lasted longer with repeated losses of blood. Healing of the necessary wounds in the operations, material lapped from these wounds, disturbance of circulation in the part fed by the artery from which blood was taken, and associated influences, combined to produce a part though only a minor share of the observed catabolic effects.

The increased elimination of the catabolic products referred to above occurred only in the urine. The amount, consistency, and composition of the faeces were apparently unaffected by the hemorrhage. Digestion did not appear to be materially disturbed at any time, even after several severe hemorrhages at short intervals. There was little or no effect on intestinal putrefaction.

Body-weight steadily declined on the original equilibrium diet after each bleeding. When the animal was allowed to eat freely, hemorrhages were followed by gradually increased weight. Moderate loss of blood markedly increased the appetite and caused thirst, even during periods when the animal was receiving an excess of food. Excessive losses of blood had temporarily an opposite effect.

<sup>1</sup> GOLL: *Zeitschrift für rationelle Medizin*, 1854, iv, p. 78.

Volume of the urine and its specific gravity fell at first after hemorrhage, then rose far above the average for several days, returning shortly to the usual quantity. With each succeeding hemorrhage, the low volume for the first twenty-four hours was slightly increased, but the cumulative rise after the first twenty-four hours became more decided and was longer continued. Hemorrhage caused an immediate stoppage of the *formation* of urine, a subsequent retardation of flow, and finally a decided stimulation. On returning the blood (defibrinated), urine immediately began to form, and flowed under special stimulating influences. Hemorrhage inhibited the hypersecretion of saliva during ether anæsthesia.

The urine was always decidedly acid in reaction before hemorrhage, slightly amphoteric occasionally. After hemorrhage, however, it was strongly amphoteric for several days.

The only observed abnormality of the urines was a transient glycosuria. This appeared to be due solely to the anæsthetic.

Effects on respiration, heart-beat, blood-pressure, and on the qualities of the blood, such as number of erythrocytes, leucocytosis, and clotting tendency, were essentially the same as those repeatedly observed by others.

After successive hemorrhages, the percentage-content of proteid and nitrogen in the blood gradually fell; that of phosphorus, sulphur, and ash remained practically stationary. Specific gravity fell, and water-content rose in each sample of blood, even when taken at very wide intervals.

*B.* Light ether anæsthesia, in these experiments, caused *decreased* elimination of nitrogen and sulphur, but *increased* output of chloride and total solids in the urine, with no special effect on specific gravity and none on phosphorus metabolism. For about twenty-four hours after light anæsthesia, urinary volume was markedly increased. After twenty-four hours there was a corresponding temporary fall below the average daily volume.

Ether caused transient glycosuria.

#### IX. BIBLIOGRAPHY.

The following references will put the reader on the main track of the literature of this subject:

ANTOKONENKO: Archives des sciences biologiques, 1893. ii, p. 516.

ASCOLI and DRAGHI: Berliner klinische Wochenschrift, 1900, xxxvii, p. 1055.

- BAUER: *Zeitschrift für Biologie*, 1872, viii, p. 567.
- BAUMANN: *Journal of physiology (English)*, 1903, xxix, p. 18.
- GÜRBER: *Münchener medicinischen Wochenschrift*, 1892, xxxix, p. 605.
- HAYEM: *Leçons sur les modifications du sang*, Paris, 1882.
- JÜRGENSEN: VON ZIEMSEN'S Handbook of General Therapeutics (translation by HAY), 1885, ii, p. 185.
- NOLL: *Ergebnisse der Physiologie (Biochemie)*, 1903, p. 433.
- SKVORTSOV: Inaugural dissertation. St. Petersburg, 1890. Cited in Bulletin No. 45, 1898, pp. 332, 340, U. S. Department of Agriculture, Office of Experiment Stations (ATWATER and LANGWORTHY).
- VOIT: HERMANN'S *Handbuch der Physiologie*, 1881, vi (i), pp. 220, 308.
- VON NOORDEN: *Lehrbuch der Pathologie des Stoffwechsels*, Berlin, 1893, p. 355.
- WILLEBRAND: *Zur Kenntniss der Blutveränderungen nach Aderlassen*, Berlin, 1900.

# THE CAUSE OF THE PHARMACOLOGICAL ACTION OF THE IODATES, BROMATES, CHLORATES, OTHER OXIDIZING SUBSTANCES AND SOME ORGANIC DRUGS.

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THIS paper contains an explanation of certain exceptions noted in my paper on the relation between physiological action and solution-tension.<sup>1</sup> It was shown in that paper that inorganic salts act on protoplasm by means of the electrical charges on the ions. The kind and degree of action was determined chiefly by the sign of the charge, whether negative or positive, and by the solution-tension or ionic potential of the ion. The chemical composition of the ion was of little importance. The poisonous power of a salt was shown to be inversely proportional to its decomposition-tension. The iodates were found to be exceptions to this law, and the action of the organic drugs, many of which do not appreciably ionize, was left unexplained. In this paper I wish to consider both these points.

The iodates have a high decomposition-tension, and, according to my hypothesis, should be inert and even less poisonous than the chlorides. I found them, on the contrary, to be quite poisonous in their action on *Fundulus* eggs, and I suggested that the iodates must have a solution-tension lower than had been supposed. During the past winter I have found that sodium iodate acts upon the motor nerve of the frog like a salt of which the anion has a high solution-tension, this ion being relatively inert.

How shall we explain this discrepancy? Upon the nerve the iodate follows the rule and is inert, acting like a salt of high decomposition-tension; upon *Fundulus* eggs it is an exception and acts like a salt of low decomposition-tension. This discrepancy is but one instance of many familiar to pharmacologists, in which marked variations have

<sup>1</sup> MATHEWS : This journal, 1904, x, p. 290.

been observed in the poisonous action of salts on the different tissues of the same animal, upon different animals, or upon the same tissue under different conditions.

The iodates belong to a class of compounds which includes the chlorates, bromates, chromates, permanganates, ferricyanides, and nitrites. All of these salts are oxidizing agents. That the physiological power of these salts is correlated with their oxidative powers is shown by a comparison of the poisonous action of the chlorates, bromates, and iodates. The minimum fatal doses for *Fundulus* eggs were as follows: for potassium iodate,  $\frac{n}{30}$ ; for the bromate,  $\frac{n}{10}$ ; and for the chlorate,  $\frac{2}{3}n$ . Dreser<sup>1</sup> states that the salts arrange themselves in this order of poisonous power for yeast, fishes, frogs, and mammals. This order, as Dreser and Binz have pointed out, is the same as the order of their oxidative powers, and these authors recognize that their pharmacological action has a casual relation with their oxidative action.<sup>2</sup> The relative oxidizing action has been particularly studied by Burchard.<sup>3</sup> If the cause of the variations in their oxidative powers were understood, we might understand the cause of the variation in the physiological action.

According to the theory of Ostwald,<sup>4</sup> an oxidizing substance is one which contains positive electrical charges with which it readily parts, or, what amounts to the same thing, one having an affinity for negative charges. The power of oxidation depends upon the ease with which the oxidizing substance gives up these charges, or upon the solution-tension, better called the ionic potential, of the cation. According to his view, therefore, all positive ions are oxidizing ions, although they differ greatly in power, and all negative ions are reducing ions.

While this hypothesis makes it easy to see how ferric chloride oxidizes, because the three-charged ferric ion gives up a positive charge with great ease, going thereby into the two-charged ferrous state; and why silver, platinum, gold, and copper salts are strong oxidizing agents, in all of which the oxidizing part is a cation of high potential, it is difficult to understand the oxidizing power of the permanganates, chlorates, iodates, bromates, ferricyanides, chromates

<sup>1</sup> BINZ: *Archiv für experimentelle Pathologie und Pharmakologie*, 1894, xxxiv, p. 204.

<sup>2</sup> BINZ: *Loc. cit.*, p. 207.

<sup>3</sup> BURCHARD: *Zeitschrift für physikalische Chemie*, 1888, ii, p. 796.

<sup>4</sup> OSTWALD: *Grundriss der allgemeinen Chemie*, 3d ed., 1899, pp. 439 ff.



and nitrites, in which the cation is without strong oxidative powers and oxidation is plainly due to that part of the molecule which, apparently at any rate, is in the negative ion.

To explain the action of the latter compounds Ostwald has made the suggestion that these oxidize, because in solutions of such salts there are present not only the ions generally supposed to be there, but also other ions of very low solution-tension. For example, the oxidizing action of nitric acid, according to Ostwald, is not due either to the hydrogen ion or to the nitrate ion, but to the fact that nitric acid dissociates in small amounts into  $\bar{\text{NO}}_2\bar{\text{OH}}$ . The oxidizing action is due to the small number of  $\text{NO}_2$  ions present, these having a very low solution-tension. When silver is dissolved in nitric acid, the reaction is to be written as follows:



In this equation the positive  $\text{NO}_2$  ions oxidize metallic silver to the ionic state. Similarly, in a solution of the permanganates, a small number of manganese ions, with a valence of seven, are present combined as the hydrate, as follows:



Although at any instant so small a number of such ions are present that they can hardly be detected, yet as soon as they are removed or used up in oxidation, more are instantly formed, thus permitting the reaction to go on very rapidly. This interpretation is supported by many well-known facts for which reference may be made to Ostwald's discussion.

#### THE IODATES.

How far may similar reasoning explain the action of the iodates? We may first consider the action of sodium iodate on potassium iodide. As is well known, no reaction takes place between these salts as long as the solution remains neutral or alkaline, but in the presence of even small amounts of acid the iodate oxidizes the iodide, setting free iodine. This reaction shows on Ostwald's hypothesis, that as soon as sodium iodate is made acid, it contains positive ions of a kind which were not present while it was neutral or alkaline, or which were present in much smaller numbers, and that these positive ions are not the hydrogen ions, but must be ions having

an ionic potential much higher than these, since hydrogen will oxidize iodine only very slowly. What are these new ions which are formed so soon as the iodate becomes acid? On Ostwald's reasoning, they should be positive iodine ions, the dissociation taking place as follows:



To test this hypothesis, I electrolyzed neutral and acid solutions of the iodate. In the neutral solution, the iodine comes off only at the anode; in the acid solution, it comes off both at the anode and cathode, but chiefly at the latter, thus indicating that in such solutions the iodine is present as a positive ion, as well as in the negative. The objection to this experiment is that the iodate is reduced at the cathode to iodide, by the nascent hydrogen formed, a reaction which really takes place, and that the acid then causes the ordinary reaction between hydriodic and iodic acids. To this objection it may be answered that the analogy between the iodates and sodium silver cyanide strongly supports the view that the separation of the iodine is, in part at least, a primary process, and that in any case there is in reality no difference in kind between primary and secondary electrolytic reactions.

Whether this explanation of the iodate action be correct or not, I wish to emphasize the fact that an iodate in an alkaline or neutral solution has different properties from an iodate in acid solution, inasmuch as in the last solution it is an intense oxidizing agent, whereas in the others it is not. Upon Ostwald's hypothesis, this variation in action of the iodate is probably to be ascribed to the fact that the character of the dissociation changes as soon as the solution becomes acid.<sup>1</sup>

We may now apply these facts to the explanation of the divergence noted in the action of the iodates on the different kinds of protoplasm. Toward *Fundulus* eggs, an iodate is a poison; toward the nerve it is relatively inert. The difference between these tissues is primarily a difference in metabolism. *Fundulus* eggs produce during division, judging from analogy with sea-urchin eggs,<sup>2</sup> large quantities of carbon dioxide, while the metabolism of the nerve is known to be very

<sup>1</sup> For indications that similar changes in dissociation occur in organic compounds, following changes in acidity, see NEF, *Liebig's Annalen*, 1899, cccix, p. 156

<sup>2</sup> LYON: *This journal*, 1904, xi, p. 52.

small. This carbon dioxide probably renders the egg-substance, or the water immediately in contact with it, far more acid than the nerve, and the result of this is an enormous increase in oxidative power of the iodate, probably due to the formation of a small number of positive ions (iodine) of very high ionic potential, which are, accordingly, very poisonous.

To make the matter more certain, that the acidity of the tissue may make the iodate greatly more active than when it is neutral, I tried the following experiment. If the gastrocnemius muscle of the frog is immersed in neutral  $\frac{m}{8}$  solution of sodium iodate, it undergoes little or no shortening for several minutes. If, however, the iodate be made very slightly acid, so that it contains about  $\frac{n}{1100}$  sulphuric acid, the muscle is quickly coagulated, causing a sharp rise in tone and rigor, preceded by fibrillary contractions. Complete coagulation ensued in about one-half an hour. To show that the hydrogen ions were not responsible for this coagulation, the other muscle of the same frog was placed in  $\frac{n}{8}$  sodium chloride of the same acidity. This muscle showed little or no shortening, and was still irritable when the other was coagulated. I observed, also, that the iodate is a much more intense poison for the muscle, which turns acid easily and produces carbon dioxide, than for the nerve.

By this explanation of the action of the iodates, it is seen that these salts, instead of being exceptions to the law stated in my former paper, confirm the hypothesis that ionic potential determines physiological action. It is, I think, highly suggestive that a slight difference in reaction of two tissues may be sufficient, if Ostwald's hypothesis be correct, to produce a difference in the ionization of salts, and cause thereby a difference in the physiological response.

#### THE CHLORATES.

The action of the chlorates, and the other salts of which the anion apparently oxidizes, may be explained in the same manner. No good explanation has been given of the peculiar difference of action of the chlorates in different animals. In the frog, *Temporaria*, and rabbits these salts are much more inert than in dogs. In the animals affected by the drug, methæmoglobin is formed, and to this the poisonous action is often attributed,<sup>1</sup> but that this is not a complete explanation of its action, I think pharmacologists generally will admit. In rabbits methæmoglobin is formed with difficulty, and the animals are much

<sup>1</sup> CUSHNY: Pharmacology, 2d ed., 1901, p. 512.

more immune than dogs. It has been suggested that in the dog the salt can penetrate the red corpuscle, and thus oxidize the hæmoglobin, while in the rabbit it cannot. In fact, dog's blood can be oxidized more easily than rabbit's. It is probable that the same factor in the blood which determines that the corpuscles of the rabbit are not dissolved by the chlorate, probably determines in the tissues their immunity to the chlorates. I have found that the laked rabbit's blood is hardly, if at all, more readily oxidized to methæmoglobin than is the unlaked blood, thus showing that a failure to penetrate the corpuscle is not the true explanation. If, however, a current of carbon-dioxide be passed through the blood of dogs or rabbits, the action of the chlorate takes place very rapidly, and methæmoglobin is quickly formed. It is, therefore, clear that while the arterial blood of the dog or rabbit is quite resistant to the action of the chlorate, the venous blood will be quickly acted on in both cases. This experiment shows that the conversion probably takes place in the body in that part of the circulation where the blood is most acid, *i.e.*, in the venous system. That the conversion of hæmoglobin to methæmoglobin is not entirely sufficient to explain the action of the chlorates, or their lack of action, is shown, also, by the fact that in *Rana Esculenta* the iodates are intense poisons, while in *Rana Temporaria* they are not; yet in the first frog, the blood does not contain methæmoglobin after their action.<sup>1</sup>

Whether rabbit's blood is more alkaline than dog's I have not observed, but the fact that the herbivorous animals secrete a strongly alkaline urine, and that their diet is one which we know by experiment increases the alkalinity of blood generally, would indicate that such was the case; the dog's urine, on the other hand, is strongly acid, and his diet of meat would increase the acidity of the blood.

If such a difference in alkalinity exists, it is sufficient to account for the greater immunity of the rabbits.

The objections raised by Cushny<sup>2</sup> to ascribing the action of the chlorates to their oxidizing action, because the chlorates are excreted for the most part unchanged, is immaterial, for the reason that a small portion of the chlorate is not accounted for, and it is probably the portion which is used up which exerts an action. The bromates and iodates are certainly changed in part, at least, to the form of halides, so that the chlorates are probably changed also.

That the pharmacological action of the chlorates, like their oxida-

<sup>1</sup> BINZ: *Loc. cit.*, p. 207.

<sup>2</sup> CUSHNY: *Loc. cit.*, p. 511.

tive action, is enormously facilitated by the presence of small quantities of acid, may be shown easily by the experiments already referred to upon the action of carbonic acid on chlorate blood. It can also be shown by allowing an  $\frac{m}{8}$  solution of sodium chlorate, slightly acidified, to run over a gastrocnemius muscle. Such a solution causes an instantaneous contraction; while the neutral chlorate, or a chloride solution of the same acidity, is inert. Upon tetanized muscle, also, the chlorate is much more poisonous than upon non-tetanized. I tried the following experiment. One sciatic nerve of a frog was stimulated intermittently for fifteen minutes. Both gastrocnemius muscles were then removed and suspended in the same solution of sodium chlorate  $\frac{m}{8}$ . The tetanized muscle underwent fibrillary contractions; its tone steadily increased; there were no large contractions; and the muscle was coagulated the next morning. The untetanized muscle underwent no shortening; it contracted rhythmically and strongly almost from the time of immersion; and the next morning was irritable and not coagulated.

From these experiments I see no escape from the conclusion already reached by Binz that the pharmacological action of the drug is determined by its oxidative action, and that this is increased by the production in any tissue of carbon dioxide, or other acid. The variations in oxidative power are due then to variations in the acidity of the tissues; the more acid the tissue, the more intense the action of the drug. We may add to Binz's conclusion the following: This increase in oxidative power is due, on Ostwald's hypothesis, to the chlorate having in it, in acid solution, positive ions of low solution-tension, which are present in the alkaline solution only in very small amount. The character of these positive ions is uncertain; but from analogy they should be chlorine ions with a valence of five. In other words, we reach the conclusion by this path, to which Binz came from other directions, that the action of the chlorate is due to its halogen atom. The fact that the acid chlorates are far less poisonous than the acid iodates is correlated with the fact that the chlorates are far less intense oxidizing agents than the iodates, as may be seen by a comparison of their actions on potassium iodide.

#### BICHROMATES AND OTHER SALTS.

The same conditions govern the oxidative action of the ferricyanides, bichromates, and permanganates. In the chromates and bichromates the oxidizing agent is the chromium ion; in the permanganates the

manganese ion; and in the ferricyanides, the ferric ion. Ostwald<sup>1</sup> has suggested for the ferricyanides that the oxidizing action is due to the three-charged ferricyanide anion going over to the four-charged state. It seems to me more probable from analogy, and the fact that the oxidizing action is more powerful in acid solution, that the action must be due to the presence of ferric ions.<sup>2</sup>

For all these compounds the following general rule may be stated: *Oxidizing salts, such as the chlorates, which have the oxidizing property connected apparently with the anion, oxidize most powerfully in acid solutions; those which have the oxidizing portion in the cation, as cupric salts, oxidize best in alkaline solutions. Pharmacologically the rule may be put as follows: All compounds of the first class act the more powerfully the more acid the tissue; all compounds of the second class act more powerfully the more alkaline the tissue.* The explanation of this rule has already been given.

As an example of the application of this rule, I believe the action of copper salts on mould spores may be cited. As mentioned in my former paper, some forms of protoplasm and particularly moulds are very resistant to copper. It is well known that the oxidative power of copper salts is much greater in alkaline than in acid solutions. For example, copper acetate in strongly acid solutions is unable to oxidize any of the sugars; if we gradually reduce the acidity, the increase in oxidative power may easily be seen. Levulose is oxidized by fairly acid solutions; as the acidity is reduced, glucose can be oxidized; then galactose; then maltose; and finally, in alkaline solution, lactose may be oxidized. It is not uninteresting that this is the order in which the body is able to utilize the sugars as foods. This experiment shows that in alkaline copper acetate, there must be present positive ions of higher ionic potential and greater power than in acid. It must therefore happen that if protoplasm is acid, copper will be relatively inert, while toward alkaline protoplasm it should be more powerful. There are reasons for believing that the protoplasm of moulds is acid, since these grow best in acid media, and Buchner and Hahn<sup>3</sup> state that the "Press-saft" of yeast is always acid, however fresh it may be.

<sup>1</sup> OSTWALD: *Loc. cit.*, p. 439.

<sup>2</sup> That the ferricyanide dissociates in part into ferric ions is indicated also by the behavior of the corresponding ferrofulminic acid salts which in acid solutions decompose completely in this way (NEF: *Proceedings Amer. Acad.*, 1894, p. 188).

<sup>3</sup> BUCHNER and HAHN: *Die Zymasegärung*, München, 1903, p. 292.

## THE ACTION OF SODIUM SILVER CYANIDE.

Another principle of pharmacological importance is brought out by a study of these oxidizing salts and of sodium silver cyanide. The latter salt dissociates chiefly into sodium and silver cyanogen ions. There are, however, a few positive silver ions present, although their number is very small so long as the solution remains alkaline. It might be supposed from the chemical behavior, that silver ions were not present, but that they are present may be shown if the solution is electrolyzed, when silver is deposited at the cathode. Practically, the whole of the silver may be thus deposited. While, therefore, at any instant there are present so few silver ions that they cannot be detected by ordinary reactions, the whole of the positive electrolysis is carried out by these minute quantities of ions. This is made possible by the fact that there is an equilibrium in the solution between  $\text{Na}^+$ ,  $\text{Ag}^+$ ,  $\text{AgCn}_2^-$ ,  $\text{Cn}^-$  ions, and  $(\text{Na}, \text{AgCn}_2)$  molecules. When one silver ion is removed by electrolysis, another is quickly generated.<sup>1</sup> It seems to me that the electrolytic behavior of this salt is in the highest degree significant for pharmacologists. It enables us to see how substances may act powerfully on protoplasm, although they are so slightly ionized that they appear not to be ionized at all. If any tissue is capable of removing silver ions from the ionic form, say by combining with them, it will act just like the cathode, and in a solution of this salt, such a tissue will cause all the silver ultimately to become active in the ionic form. Furthermore, if the conditions in any tissue favor the formation of such ions, more than in another tissue, and if the tissue can combine with such ions, as all tissues can, that tissue will appear to exert a selective action on the silver; it will pick it out and be poisoned by the salt more than another tissue. Such conditions may easily prevail, for, as stated, it is only necessary to decrease the alkalinity or increase the acidity to increase greatly the number of silver ions. Sodium silver cyanide, in other words, is an oxidizing agent like the iodates, only instead of splitting off positive iodine, it splits off positive silver. The fact that the silver cyanides split off small numbers of the silver ions strongly confirms the hypothesis that the iodates and chlorates dissociate small numbers of the iodine and chlorine ions.

Organic chemistry is full of instances showing the importance of

<sup>1</sup> OSTWALD: *Loc. cit.*, p. 473.

these substances formed as intermediate products of reactions, substances which may be present at any instant only in minute traces, and yet a large quantity of a reacting substance may pass with great rapidity, molecule by molecule, through such stages.<sup>1</sup> I believe the application of this principle will ultimately harmonize the action of the organic drugs with the hypothesis that inorganic and organic salts act on protoplasm by the electrical charges on the ions, the degree of action being determined by the number and ionic potential of these ions.

Two or three instances will suffice to indicate this more precisely. Amyl nitrite and sodium nitrite produce nearly the same physiological result. Sodium nitrite in acid solutions is a strong oxidizing agent. It acts pharmacologically not by means of its sodium ions or its negative nitrite ions, which are present in large numbers, but undoubtedly, from what has been said of the relation of pharmacological to oxidizing action, by means of small quantities of positive NO ions its solutions contain. These ions have a very high ionic potential, and cause thereby an intense oxidation and pharmacological action. Such ions will be set free in the more acid tissues. I have found that the nitrite is relatively inert toward alkaline tissues, but poisonous toward acid. Now amyl nitrite does not dissociate electrolytically, except very slightly, nevertheless there is reason for thinking that it does dissociate somewhat because it will break up in water with the formation of amyl alcohol, and it is easy in reactions to replace the nitrite group, showing it to be loosely bound. Besides this, tertiary amyl iodide is known to split very rapidly in water into tertiary amyl alcohol and hydriodic acid. If amyl nitrite does dissociate these same NO ions, as its chemical behavior indicates, it is clear that it must exert a similar action to the inorganic nitrites.<sup>2</sup> For even though at any time there are present but small amounts of such ions, so great is their combining power that they are at once removed by the tissues, and new ions are generated to take their place. Amyl nitrite, or any nitrite capable of this dissociation, must produce the same pharmacological effect.

The second instance is chloroform. Chloroform in all its physio-

<sup>1</sup> NEF has particularly emphasized the importance of these dissociated molecules of organic compounds, both for chemical and physiological reactions (see Liebig's *Annalen*, 1899, cccix, p. 156; 1901, cccxviii, p. 2).

<sup>2</sup> BINZ: *Archiv für experimentelle Pathologie und Pharmakologie*, 1880, xiii, p. 133.



logical reactions bears a remarkable resemblance to atomic chlorine, as has been pointed out by Binz. Negative chlorine is a stimulant, but atomic chlorine, according to the theory, is positively charged, and must hence have an opposite or depressant action. Chlorine gas is an intense oxidizing agent. This shows, it seems to me, that it must contain positive ions of high potential, although in small quantities. Now chlorine gas acts on protoplasm, as Binz has shown, as an anæsthetic.<sup>1</sup> Frogs are anæsthetized by it; nerves lose their irritability; muscles exposed to its vapors undergo a prolonged tetanic rigor exactly similar to that produced by acids or chloroform or other anæsthetics. Both chloroform and chlorine are intense irritants to skin and mucous membranes, although the chlorine is the more powerful. The sole difference in action is a difference in degree.

Evidence is not lacking that chloroform does dissociate small quantities of chlorine ions. The fact that methyl chlorides do ionize, though slowly, is shown by their behavior. Hydrolytic decomposition takes place in small amounts, as follows:



The halogen atoms in such compounds react slowly with silver nitrate, this is particularly true of the iodides, forming silver halides. After chloroform is given, the chlorides are increased in the urine,<sup>2</sup> though the chlorine is not derived wholly from the chloroform. When ethyl bromide is given, bromides appear in the urine.<sup>3</sup> Methyl and ethyl bromides and iodides react in the test tube more rapidly with silver nitrate than do the chlorides,<sup>4</sup> and they are more intense depressants than the chlorides.<sup>5</sup> The iodine and other halogens in the ethyl compounds are less easily split off than the same halogens in the methyl compounds,<sup>6</sup> and corresponding with this the ethyl chlorides and bromides are less strongly anæsthetic, molecule for molecule, than the methyl.<sup>7</sup> The pharmacological action of

<sup>1</sup> BINZ: *Archiv für experimentelle Pathologie und Pharmakologie*, 1880, xiii, p. 139; 1894, xxxiv, p. 199.

<sup>2</sup> KAST: *Zeitschrift für physiologische Chemie*, 1888, xii, p. 277.

<sup>3</sup> BINZ: *Archiv für experimentelle Pathologie und Pharmakologie*, 1891, xxviii, p. 201.

<sup>4</sup> MEYER and JACOBSON: *Lehrbuch der Organischen Chemie*, I, p. 183.

<sup>5</sup> OVERTON: *Studien über die Narkose*, Jena, 1901, p. 103.

<sup>6</sup> MEYER and JACOBSON: *Loc. cit.*

<sup>7</sup> DIEBALLA: *Archiv für experimentelle Pathologie und Pharmakologie*, 1894, xxxiv, p. 137; OVERTON: *Studien über die Narkose*, Jena, 1901, p. 103.

chloroform is in every respect that of a substance forming positive ions of high potential. It is immaterial whether the chlorine or the methyl or methylen (Nef) is the negative or positive ion. In any case it is the positive ion which predominates. Inasmuch, however, as the  $\text{CH}_3$  ion, if formed, must be of much lower solution-tension than hydrogen, it is probable, I think, from the law of mass action, and the relative solution-tensions of chlorine and methyl, that the chlorine will be dissociated in part, at least, as a positive ion.

The hypothesis of the decomposition of chloroform is confirmed, also, by the action of chloroform on albumin. Albumin is coagulated by acids, or strong positive ions. It is, for example, coagulated by any strong acid, but particularly by nitric acid, which contains the powerful positive  $\text{NO}_2$  ions. It is precipitated by bromine, chlorine, or iodine *in statu nascendi*, that is, by the positive halogens. It is precipitated by the bichromates and other oxidizing agents in acid solution. It is also precipitated by chloroform. If we turn to other organic haloids, similar relationships are seen. Glycerine does not precipitate albumin; monochlorhydrin precipitates it very little or not at all. It coagulates muscle very slowly, and is a very weak anæsthetic. Dichlor- and trichlor-hydrin precipitate albumin readily and are more powerful anæsthetics.<sup>1</sup>

The third instance is ammonium hydrate. In my opinion, the pharmacological action of this substance cannot possibly be referred either to the  $\text{NH}_4$  or the  $\text{OH}$  ions its solutions contain. That the  $\text{NH}_4$  ion is not responsible for its action, is shown by the fact that ammonium chloride is not particularly poisonous for many forms of protoplasm. Similarly, the few hydroxyl ions present in the hydrate solutions cannot be made responsible for its intense action. This action is extraordinary. A very minute amount of the vapor causes muscle to shorten quickly, and go into a rigor, accompanied by coagulation. Exactly the same effects are produced by strong acids, and the anæsthetics. It is an intense poison also for many eggs, *i. e.* Arbacia (Lyon). It is quite evident, I think, that the pharmacological action of this compound is due to compounds (ions) present in very small numbers, but intensely active. It is not impossible that there is some hydroxylamine present, or possibly a dissociation of  $\text{NH}_3$  into  $\text{NH}_2$  and  $\text{H}$ . Whatever these ions may be, the pharmacological action is, I think, due mainly to them, and not to the obvious ions  $\text{NH}_4$  and  $\text{OH}$ .

<sup>1</sup> MARSHALL and HEATH: Journal of physiology, 1897, xxii, p. 38.

There is hence good reason for thinking that both the nitrites and chloroform, and probably other drugs as well, do dissociate slightly; that their physiological action is, roughly at any rate, proportional to their chemical activity, which is, in its turn, greatest in those which decompose into ions most easily; and, since the physiological effects are identical with those produced by ions, we may conclude, as has already been concluded by Binz, that they act by means of these dissociation products, or, as we now call them, ions.

Other examples might be given to illustrate these general principles. If they are true, it follows that the organic drugs owe their action to ions of very high ionic potential, generally present in small amounts at any one time, but formed readily and formed in different amounts under slightly different conditions. The chemical composition of these ions is of no importance, as has already been stated in my former paper;<sup>1</sup> it is their ionic potential, sign and numbers which determine their action. The instances cited will suffice to show the importance of these two generally neglected pharmacological principles: *i. e.*: (1) *changes in the kind of dissociation due to changes in the reaction of the tissues*; and (2) *the importance of ions (dissociation products) present in minute traces, but in equilibrium with other ions and molecules.*

What causes the dissociation to change in acid solutions? This can only be conjectured, but I believe the fact that it does change indicates the truth of the suggestion of Richards<sup>2</sup> that the atomic volume and its correlate solution-tension varies with the solution-tension or power of the oppositely charged ion present. I drew such a conclusion from the physiological behavior of the salts. It may be that in the presence of the very positive ion sodium, the iodine in an iodate is more negative than is usual. Its solution-tension is higher. It remains almost altogether in the anion. In the presence, however, of the weak positive ion hydrogen, the iodine is not so negative; its solution-tension for the negative charge falls, and a part of the iodine actually takes a positive charge, so that there are present a few positive iodine ions. While this hypothesis may be altogether wrong, the facts may be interpreted in harmony with it. It remains to be seen whether it can be harmonized with the theory developed by Nernst for concentration chains.

<sup>1</sup> This disposes of the objection raised against Binz's explanation of the chloroform action, that many anæsthetics contain no halogens.

<sup>2</sup> RICHARDS: *Zeitschrift für physikalische Chemie*, 1902, xl, p. 172.

# THE INFLUENCE OF THE STROMATA AND LIQUID OF LAKED CORPUSCLES ON THE PRODUCTION OF HÆMOLYSINS AND AGGLUTININS.

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I HAVE previously shown<sup>1</sup> that colored corpuscles laked in certain ways (by foreign serum, freezing and thawing, heating, standing for a time either under septic or aseptic conditions) lose their hæmoglobin completely without any marked increase taking place in the electrical conductivity of the blood or of a suspension of corpuscles in salt solution. Other laking agents, like saponin (in more than strictly minimal doses) and water, cause an absolute or relative increase of conductivity, accompanying the exit of the hæmoglobin. A similar increase in conductivity is caused by the addition of agents of the second group to the ghosts or shadows of corpuscles laked by any one of the first group. The simplest explanation of these facts is that the hæmoglobin is discharged under the influence of laking agents of the first group, without any important escape of electrolytes, while the second group causes, in addition, and perhaps subsequent to the discharge of the hæmoglobin, such an alteration in the stroma as leads to the escape of electrolytes as well. I have been endeavoring in various ways to throw light upon the nature of the change in the stroma or envelope associated with hæmolysis. Here will be described briefly some experiments, which I hope to continue, on the effect of injection of the stromata and of the substances which escape from the corpuscles respectively, in hæmolysis caused by various laking agents, as regards the production of specific agglutinins and lysins. It seemed not impossible that quantitative or even qualitative differences might be detected in this respect, depending

<sup>1</sup> STEWART: *Journal of physiology*, 1899, xxiv, p. 211.

on the method of laking, and especially differences between the products of laking by the two groups of hæmolytic agents mentioned. For the escape of electrolytes caused by the second group suggests that they bring about a more profound alteration in the stroma than the first.

In connection with this investigation the question was also studied, whether formaldehyde-fixed corpuscles, which have been shown<sup>1</sup> to be still susceptible in certain respects to the action of some laking agents, have any power of causing, on injection into an animal, the production of specific bodies similar to those formed after injection of fresh corpuscles. Since saponin, for instance, causes in formaldehyde-fixed corpuscles an increase in conductivity as great as that produced by it in fresh corpuscles, certain constituents of the corpuscles may be supposed not to be fixed, or at any rate not to be fundamentally altered by the formaldehyde; and it was of interest to determine whether, among these the substances which on injection into an animal of another species give rise to the production of agglutinins and hæmolysins (the agglutininogens and hæmolysinogens or, more briefly, lysinogens), might not be included.

Not a great many experiments have previously been made on the question whether the ghosts or the liberated contents of laked corpuscles contain agglutininogens or lysinogens, and, so far as I am aware, the few which have been made have all been on corpuscles laked by water, an agent which, according to my observations, is perhaps, on account of the violence of its action, less suitable than any other for use in observations concerning the normal distribution of these substances in the corpuscle. It is also more difficult, in the case of mammalian corpuscles at any rate, to obtain considerable quantities of ghosts free from hæmoglobin with water-laking, than when other agents are used.

Bordet<sup>2</sup> states that the injection of water-laked rabbit's stromata, free from hæmoglobin, into the peritoneal cavity of a guinea-pig caused the production of hæmolysin which was specific for rabbit's corpuscles. The injection of the hæmoglobin-containing liquid gave a negative result.

V. Dungern<sup>3</sup> came to the opposite conclusion. He laked bird's cor-

<sup>1</sup> STEWART: *Journal of physiology*, 1901, xxvi, p. 470.

<sup>2</sup> BORDET: *Annales de l'Institut Pasteur*, 1900, xiv, p. 257; quoted by ASCHOFF: *Zeitschrift für allgemeine Physiologie*, 1902, i. p. 156.

<sup>3</sup> VON DUNGERN: *Münchener medicinische Wochenschrift*, 1899, xlv, p. 449.

puscles by water, and hastened the separation of the shadows by shaking up with a little ether. He then washed the shadows on a filter with ether-containing water till all the hæmoglobin was removed. The shadows were injected into the peritoneal cavity of one guinea-pig and the hæmoglobin-containing liquid into another. After ten or eleven days he injected into the peritoneal cavity of each guinea-pig some normal bird's corpuscles. In neither case was any hæmolysis of the normal bird's corpuscles produced. He therefore asserts that the substance in the corpuscles which gives rise to the hæmolysin in the serum of guinea-pigs injected with intact bird's corpuscles, is not contained in the shadows, nor is it the hæmoglobin. It is to be noted, however, that this author not only employed a violent laking agent (water), but also caused further changes in the stromata by washing them with ether, which is itself a laking agent, and possesses the power of dissolving out substances like cholesterin and lecithin, not only from stromata, but from fully fixed formaldehyde-hardened corpuscles. His results, therefore, are not calculated to throw much light on the normal distribution of the lysinogenic substance in the intact corpuscle.

P. Nolf<sup>1</sup> injected the stromata of water-laked chicken's corpuscles intraperitoneally into rabbits. The stromata were washed with salt solution. Into other rabbits the hæmoglobin-containing liquid was injected. After a sufficient interval had elapsed, he examined the agglutinating and hæmolytic power of the rabbit's serum for intact chicken's corpuscles. The serum of the rabbits which had received stromata caused strong agglutination, but comparatively little hæmolysis. The serum of the rabbits injected with the liquid was strongly hæmolytic, but had comparatively little agglutinating power. He concluded that the stroma constituents only give rise to the production of agglutinin; the "cellular contents," only to the production of the specific hæmolytic antibody (intermediary body of Ehrlich). This "diagrammatic" result, the sharpness and simplicity of which are only attained by assuming that the relatively feeble lysinogenic action of the shadows, and the relatively feeble agglutininogenic action of the liquid, are due to mutual contamination, is perhaps not unconnected with this observer's "diagrammatic" conception of the structure of the corpuscle, which he looks upon as a vesicle containing hæmoglobin in solution. A complete topographic separation of

<sup>1</sup> NOLF: *Annales de l'Institut Pasteur*, 1900, xiv, p. 297.

the active substances in the corpuscle will appear far less likely, if the stroma be regarded as a mass of protoplasm in whose meshes the hæmoglobin is contained, or to some of whose constituents it is united.

I desire to avoid the term "cellular contents," which Nolf applies to the constituents of the corpuscle discharged in water-laking, as it implies that the whole contents of the corpuscle, except the stroma, escape unaltered, and that the stroma contributes nothing whatever to the extruded liquid. This may, perhaps, be the case in the gentler kinds of laking, but is unlikely to be true in water-laking. I prefer, therefore, to speak of the solution of ejected substances as the extra-corpuscular liquid, or the hæmoglobin-containing liquid, or, for the sake of brevity, where no ambiguity can arise, simply as the liquid.

My experiments were all made on guinea-pigs and rabbits. The guinea-pigs were injected intraperitoneally or subcutaneously with the stromata and liquid, respectively, of rabbit's laked corpuscles. In one case a guinea-pig was treated with dog's blood which had been allowed to undergo autolysis for three years. The rabbits were injected subcutaneously with the stromata or liquid of dog's corpuscles. Before laking, the corpuscles were thoroughly washed with 0.9 per cent salt solution, and after laking the ghosts were washed with the same liquid. When water-laking was employed, the separation of the stromata was facilitated by the addition of a sufficient amount of sodium chloride in substance, or of a 9 per cent solution of it to make the strength of the liquid in which the ghosts were suspended approximately that of a 0.9 per cent solution. The salt solutions used for washing the corpuscles and ghosts, and the water used for laking, were sterilized by boiling. The injections were made with aseptic precautions. Control experiments were done in which unlaked washed rabbit's corpuscles were injected into a guinea-pig, and intact washed dog's corpuscles into a rabbit. The sera obtained from these animals furnished a standard with which to compare the other sera, as regards intensity of action. It must, however, be remarked that quantitative results obtained in this way can only be approximately accurate, unless indeed such a large number of experiments is performed as is necessary to eliminate the influence of idiosyncrasy. Further, owing to the fact that the injections, for various reasons, could not all be made at the same time, nor the specimens of blood be all obtained after the same interval, control serum was not always available. Although, as is well known, the most active sera are

obtained after repeated injections, I purposely refrained in most experiments from giving more than one, with the idea that if, as Nolf supposed, the appearance of some agglutinin after injection with liquid and of some lysin after injection with stromata is due to mutual contamination, the secondary product might be present in insignificant amount. As a consequence, rather larger quantities than usual of the serum to be tested were employed. The single injection was usually large, and the reaction was unmistakable. In these experiments I thought it well first to establish the presence or absence of a marked hæmolytic reaction without attempting to show in each case that the hæmolytic power was due to a specific intermediary body. In one animal, however, it was proved that an intermediary body had been produced (guinea-pig J (8) to (12)). In further experiments now going on this point will be examined more closely.

In all, nineteen guinea-pigs, weighing from 500 to 850 grammes, and five rabbits, weighing from 2 to 3 kilos, received injections. Of the guinea-pigs, nine survived, or were allowed to live, only two days or less, as they rapidly developed toxic symptoms. These will be referred to briefly later on. One guinea-pig lived only five days. The remaining nine and the five rabbits furnished the material from which the conclusions as to the specific agglutininogenic and lysinogenic power of the stromata and liquid, respectively, are entirely drawn. With one exception, only the protocols of these experiments are reproduced.

Of the nine guinea-pigs which survived indefinitely, or at any rate long enough to provide suitable material, one (A) received the stromata of rabbit's corpuscles laked by dog's serum; one (B), heat-laked rabbit's stromata with some of the extra-corpuscular liquid; one (F), normal washed rabbit's corpuscles; one (H), washed rabbit's corpuscles incompletely fixed with formaldehyde; one (I), the extra-corpuscular liquid of rabbit's corpuscles laked by alternate freezing and thawing; one (K), the washed stromata of the same corpuscles laked by freezing and thawing; one (J), the extra-corpuscular liquid of rabbit's corpuscles laked by heat; one (J'), the washed stromata of the same heat-laked corpuscles; and one (O), the extra-corpuscular liquid of rabbit's corpuscles laked by the minimal quantity of water necessary for complete discharge of the hæmoglobin.

Of the five rabbits, one (A) received washed dog's corpuscles, fully fixed with formaldehyde; one (B), the washed stromata of dog's corpuscles laked with excess of water; one (C), the liquid from the same



water-laked dog's corpuscles; one (D), normal washed dog's corpuscles; and one (E), the washed stromata of dog's corpuscles laked by saponin (minimal dose). The choice of the material injected into the rabbits was determined by the desire to fill, as far as possible, the blanks in the series caused by the rapid death of so many of the guinea-pigs. Rabbit C was seen to be dying on the ninth day after injection, and was killed. Rabbit D had also developed serious symptoms by the fourteenth day, and was then killed. The remaining three survived indefinitely.

The results for the nine guinea-pigs and five rabbits summarized in Table I seem to justify the following conclusions:

1. Both the stromata and the hæmoglobin-containing liquid of colored corpuscles laked by water, by freezing and thawing, and by heat, possess the power of causing the production of sera with specific hæmolytic and agglutinating power, when injected into an animal of a different species. The same is true of the stromata of saponin-laked corpuscles,<sup>1</sup> and of corpuscles laked by foreign serum.<sup>1</sup>

2. The injection of the stromata of corpuscles laked by the milder agents (heat, freezing and thawing, foreign serum) causes the production of sera whose agglutinating power is more marked than their hæmolytic power. The injection of the stromata of corpuscles laked by a minimal dose of saponin has the same effect.

3. The injection of the hæmoglobin-containing liquid after laking by the milder agents (heat, freezing and thawing) causes the production of sera whose hæmolytic power is more conspicuous than their agglutinating power, when compared with the sera produced after injection of the corresponding stromata.

4. The injection of the stromata of water-laked corpuscles causes the production both of specific agglutinin and of specific hæmolysin. The same is true of the hæmoglobin-containing liquid. But the serum produced under the influence of the stromata is weak in agglutinating power, and that produced under the influence of the liquid strong in agglutinating power, in comparison with the sera produced under the influence of the stromata and liquid, respectively, of corpuscles laked by the milder agents. On account of the difficulty of completely freeing the stromata of non-nucleated corpuscles from hæmoglobin after water-laking, more experiments are required on this point, and these are now going on.

<sup>1</sup> The hæmoglobin-containing liquid was not tried in these cases, as it might be expected to produce direct toxic effects.

5. The injection of corpuscles fully fixed by formaldehyde causes the production of serum with specific agglutinating and hæmolytic powers, but the agglutinating action is more marked than the hæmolytic. The injection of corpuscles partially fixed by formaldehyde, but still capable of being slowly laked by water, causes the production of a serum with strong specific hæmolytic, as well as strong agglutinating, power.

6. The lysinogen, like the specific hæmolysin (intermediary body) to which it gives rise, and unlike the complement, is relatively thermostable, not being destroyed by the temperature of heat-laking (60°-62°). The same is true of the agglutininogen.

The most reasonable assumption which will explain the above results is that the corpuscles contain agglutininogens and lysinogens partly in the form of a relatively firm combination in the stroma, and partly in solution or loosely bound to the stroma. The agglutininogens seem to be in the main firmly united with the stroma, and the lysinogens in the main more loosely united, or perhaps in part dissolved in the "cell contents." When the corpuscles are laked by the milder agents, lysinogens easily escape from them in considerable amount, agglutininogens in comparatively small amount. When the corpuscles are laked by water, which causes more profound changes in the stroma, more of the agglutininogens may be supposed to be liberated.

The fact that formaldehyde-hardened corpuscles, in which the hæmoglobin is completely fixed, give a positive agglutininogenic reaction lends support to the view that the agglutininogens are largely in the stroma or envelope. Since the lysinogenic power of corpuscles partially fixed by formaldehyde is greater in proportion to the agglutininogenic power than in fully fixed corpuscles, we may conclude that the lysinogens are sooner and more completely altered by formaldehyde than the agglutininogens. This agrees with the idea that the lysinogens are in a different condition from the agglutininogens, and perhaps less closely associated with the stroma.

Nicolle and Trenal<sup>1</sup> have expressed the view that in bacteria the power of being agglutinated and the power of producing an agglutinin always reside together, every agglutinable microbe being capable of causing agglutination, and every non-agglutinable microbe being incapable of causing agglutination. Since formaldehyde-fixed

<sup>1</sup> NICOLLE and TRENEL: *Annales de l'Institut Pasteur*, 1902, xvi, p. 562.

corpuscles still possess the power of causing the production of specific agglutinins when injected into animals, we might expect that they would still be capable of being agglutinated by specific sera. This is the case, as is illustrated in Tables II and III. In the agglutination of formaldehyde-fixed corpuscles by specific serum, while clumping was very distinct, under the microscope, and the agglutinated corpuscles sank rapidly to the bottom of the test-tube, the agglutination was more easily broken up by shaking, than in unfixed corpuscles agglutinated by the same serum (after heating to 60° to prevent hæmolysis). Aggregation without increase of viscosity of the corpuscles seemed to be the characteristic phenomenon. This is in favor of the view that the agglutination of normal corpuscles is in part, and perhaps essentially, due to a tendency to aggregation produced by a change in the colloids of the corpuscle, not necessarily accompanied by an increase in the viscosity of the envelope. That aggregation and the actual sticking together of the corpuscles are not occasioned by the same agency is perhaps further illustrated by the fact, previously pointed out by me,<sup>1</sup> that saponin, which in minimal dose causes agglutination of unfixed corpuscles and their ghosts, the ghosts forming a mass at the bottom of the tube, which can only be shaken up with difficulty, produces on formaldehyde-fixed corpuscles an effect the opposite of agglutination, the corpuscles remaining suspended longer, and being shaken up more easily than in the absence of saponin. Now saponin certainly brings about some change in the envelopes of formaldehyde-fixed corpuscles, as is shown by the marked increase of conductivity which it causes. If this action is similar to that which produces agglutination in the unfixed corpuscles, the consummation of the agglutinative process may be prevented by some inhibitory effect of the saponin on aggregation of the corpuscles after the preliminary changes in the envelope have been accomplished. No change in the conductivity of a suspension of dog's formaldehyde corpuscles was produced by the agglutinative serum of Rabbit D.

Putting everything together, we may pretty safely conclude that the property of the corpuscle on which agglutination by specific sera depends, and which is not destroyed by formaldehyde, is a property of the envelope or stroma, in which also the agglutininogenic power of the corpuscle must, mainly at any rate, be supposed to reside.

The fact that formaldehyde-fixed corpuscles also possess lysinogenic

<sup>1</sup> STEWART: *Journal of experimental medicine*, 1902, vi, p. 259.

power seems to indicate that the stroma contains lysinogens which are not destroyed by formaldehyde, unless, indeed, we suppose that the precipitated methæmoglobin produced from the hæmoglobin is still lysinogenic. But, so far as I am aware, it has never yet been demonstrated that even crystallized oxyhæmoglobin has any lysinogenic action. Much unaltered oxyhæmoglobin was still present in the autolysed blood injected into Guinea-pig G, yet the reaction was negative as regards the serum of the guinea-pig, although the liquid from the thoracic cavity showed some hæmolytic power. The experiment, however, which was made for the purpose of investigating the action of blood submitted to autolysis, and not of hæmoglobin, is inconclusive, as the animal only lived five days.

It would be interesting to determine the effect on the agglutinogenic and lysinogenic power of formaldehyde-fixed corpuscles, of acting on them before injection with saponin, bile salts, and perhaps other laking agents, including biological hæmolysins, and of extracting them with ether and other solvents. The effect of other fixing agents than formaldehyde, and of temperature on the agglutinogenic and lysinogenic activity of corpuscles, ought also to be studied. The effect of injections of the ghosts of corpuscles laked by the milder agents, and then acted on by the more violent ones, which cause them to lose electrolytes, might also throw light upon the nature and distribution of the agglutininogens and lysinogens. It is to be expected that other animal cells than red corpuscles and bacteria may still preserve some power of causing the production of agglutinins, lysins, or antitoxins after fixation with formaldehyde, or perhaps with other reagents. The practical aspects of such a possibility are not to be overlooked. The investigation of the effects of saponin, ether, etc. on formaldehyde-fixed corpuscles, in regard to their power of being agglutinated by specific sera, might also throw light on the mechanism of agglutination. The fact that the Widal-Grünbaum reaction, as Ficker has shown,<sup>1</sup> succeeds with typhoid bacilli after they have been acted on by a preservative liquid, is suggestive, as it is a phenomenon analogous to the agglutination of formaldehyde-fixed red corpuscles by specific serum. Ficker has refrained from publishing his process, so far as I am aware. It would be interesting to see whether formaldehyde would not be a suitable "preservative."

<sup>1</sup> FICKER: *Berliner klinische Wochenschrift*, 1903, xl, p. 1021. See also MEYER: *Ibid.*, Feb., 1904; and RADZIKOWSKI: *Wiener klinische Wochenschrift*, 1904, p. 276.

One or two incidental observations are perhaps worthy of notice. In water-laking of blood or washed corpuscles, as Nolf<sup>1</sup> has pointed out, the last trace of hæmoglobin is not very readily removed by washing from the ghosts of chicken's corpuscles. I have observed that this is the case in dog's corpuscles even when a very considerable excess of water is employed in laking, *e.g.*, twenty volumes of water to one volume of blood. If the ghosts are separated by centrifugalizing after the addition of sodium chloride in substance, or in strong solution till the strength of the mixture is about that of a 0.9 per cent sodium chloride solution, the ghosts form a markedly red sediment. If the hæmoglobin-containing liquid be removed, and excess of water again added to the ghosts, they lose some more of their hæmoglobin, but only with difficulty, and even after several washings, the addition of sodium chloride being employed in each case to hasten their separation, they still contain some blood-pigment. Two possible explanations suggest themselves. (1) The swollen ghosts may be simply filled with a solution of hæmoglobin of the same strength as the extra-corpuscular liquid, the hæmoglobin passing freely through the distended envelope in both directions, although obviously in regard to sodium chloride the envelope must act as a semi-permeable membrane, else the ghosts would not shrink on addition of that substance. If now when the ghosts shrink the hæmoglobin in solution in their interior is unable to pass out through the envelope, they will, of course, be more deeply tinged than the extra-corpuscular liquid. (2) The other and more likely explanation is that the stroma, while readily parting with the main portion of the hæmoglobin, retains a residue, which is either in firmer combination with it than the rest,—in some such condition, for example, as the hæmoglobin of striped muscular fibres, or is perhaps mechanically entangled in it by the precipitation of globulins by the water. To determine whether stromata, once completely deprived of hæmoglobin, would take it up from solution, and retain it when caused to shrink, blood-corpuscles were laked with water, and washed repeatedly till free from hæmoglobin. They were then added to the hæmoglobin-containing liquid of water-laked blood or washed corpuscles after removal of the ghosts. The mixture was allowed to stand over night, and the stromata were then separated by the centrifuge after addition of sodium chloride. They did not appear to contain any hæmoglobin.

Another experiment was made in the following way. Dog's blood

<sup>1</sup> NOLF: *Loc. cit.*

was laked by water. The stromata was separated as well as possible by centrifugalization, without the addition of sodium chloride. The supernatant liquid was pipetted off. It still contained ghosts, but not so many as the bottom of the tubes. To a given volume of the supernatant liquid, a known amount of sodium chloride solution was added, and the mixture was labelled A. To the suspension of stromata from the bottom of the tubes sodium chloride solution was added in the same proportion, and the mixture labelled B. A and B were now centrifugalized. The supernatant liquids of A and B contained the same proportion of hæmoglobin, as estimated colorimetrically. Now if the ghosts in shrinking had retained all the hæmoglobin in solution in them while the water passed out, the supernatant liquid of B ought to have been weaker as regards hæmoglobin than that of A, since the ghosts in B made up a greater part of the total volume of the suspension than the ghosts in A. The hæmoglobin in ordinary solution in the ghosts must therefore have passed out of them, as they shrank, in the same proportion as the water. The stromata after separation from A and B were distinctly red, which can only be explained in accordance with (2).

It is much more difficult to completely free dog's water-laked ghosts from hæmoglobin by repeated washings with salt solution, than it is to free chicken's ghosts, because, possibly on account of their being ballasted by the nuclei, the chicken's ghosts can be quantitatively recovered after each washing. In the case of the dog's ghosts, there is always great loss.

The protective influence of the serum of a given kind of blood on its own corpuscles, against the action of a specific hæmolytic serum, has often been observed in the course of this work, for instance in Rabbit A, Experiments (3) and (4), March 15, in Rabbit C, (3) and (5), in Guinea-pig K, (1) and (2), (7) and (11).

The fact that the serum of the foetus need not share in the specific hæmolytic and agglutinating properties of the serum of the mother is well illustrated in Guinea-pig K, Experiments (8), (9), and (10). While the serum of the mother, which had been injected with the stromata of rabbit's corpuscles laked by freezing and thawing, caused laking and good agglutination in rabbit's corpuscles, the serum of the embryos had no such effect. The amniotic and allantoic liquids were likewise without action (K, (4), (5), (12), and (13)).

The foetal blood-corpuscles in this case were scarcely laked by rabbit's serum, while the maternal corpuscles were rapidly laked.

In one instance (Rabbit C, (8) ) heating to 60° abolished the agglutinative action of a specific serum for dog's corpuscles, in the presence of dog's serum, but not for washed dog's corpuscles. The specific serum was obtained from a rabbit which had received an injection of the hæmoglobin-containing liquid of dog's water-laked corpuscles.

It was observed that the serum from two guinea-pigs, which, so far as was known, were normal and had not received injections of foreign blood, caused laking and agglutination of normal rabbit's washed corpuscles (B, (3), (4), (12), (13) ), although the action was less marked than in the case of the artificially produced specific serum. The serum of the normal guinea-pigs did not lake or agglutinate normal rabbit's corpuscles in the presence of rabbit's serum.

The attempt was made to determine whether and when, in aseptic autolysis of blood, the agglutininogen and lysinogen are so altered as to lose their activity (Guinea-pig G). Dog's blood which had been kept for nearly three years in sealed tubes was injected into a guinea-pig. Unfortunately the animal only survived five days. As the blood had clotted in the vessels, only a little blood-serum considerably diluted with salt solution, could be obtained, and this gave a negative result with dog's corpuscles. Some liquid which was removed from the thoracic cavity of the guinea-pig, and which was mixed with blood, gave a positive hæmolytic reaction with dog's corpuscles. So that, although it would be unjustifiable to draw conclusions from such an experiment, the possibility may be admitted, that even after that long interval the lysinogenic substance had not been destroyed. If hæmoglobin has lysinogenic powers, this would receive an easy explanation. As soon as material has been prepared, I hope to repeat this experiment, and to extend it so as to embrace an examination of the effect of autolysis of blood or blood-serum on the production of precipitins.

A few words may be said about the guinea-pigs which died or were killed, two days or less after injection. Of these one (C) received the washed corpuscles of 5.5 c.c. of rabbit's blood; one (D), the stromata of 6 c.c. of the same rabbit's blood, laked by freezing and thawing, with a small amount of the hæmoglobin-containing liquid. The injection in D was intraperitoneal. In less than forty-eight hours the guinea-pig was dead. Under the skin there was much œdema liquid containing hæmoglobin, and having a fairly strong hæmolytic action on washed rabbit's corpuscles, and also on the blood of Guinea-

pig E. The diluted blood-serum of D, obtained by rubbing up clot from the heart in sodium chloride solution, had also a fair laking action on rabbit's corpuscles. A guinea-pig (E), which received the washed stromata of rabbit's washed corpuscles (corresponding to 5 c.c. of blood), laked by heating to  $55^{\circ}$ , and then to  $61^{\circ}$ , and a further injection on the following day, corresponding to 3 c.c. of rabbit's blood, died in less than forty-eight hours from the first injection. There was some œdema liquid, not containing hæmoglobin, under the skin; also some liquid in the peritoneal cavity. The peritoneal liquid, when added to washed rabbit's corpuscles, and put at  $30^{\circ}$  C., clotted to a firm jelly, and caused considerable laking of the corpuscles. The blood-serum, diluted with sodium chloride solution, was not lytic for rabbit's corpuscles.

A small guinea-pig (H') received intraperitoneally the mixed ghosts and extracorporeal liquid of a suspension of washed rabbit's corpuscles (corresponding to 5 c.c. of blood), laked by a trace of saponin in substance so small that it only caused complete laking in fifteen minutes at  $40^{\circ}$ . In a little more than twenty-four hours it was dead. There was much red liquid under the skin of the abdomen. The muscles in this region had a sodden and semi-digested appearance. The liquid caused good laking of dog's and rabbit's washed corpuscles, which was abolished by heating it to  $59^{\circ}$  for fifteen minutes. The dilute blood-serum obtained by rubbing up the partially clotted blood in the heart with sodium chloride solution caused no hæmolysis either of rabbit's or dog's corpuscles.

A guinea-pig (L) received intraperitoneally the washed ghosts of rabbit's washed corpuscles (corresponding to 10 c.c. of blood), laked by a small dose of saponin in substance. There was a very large sediment of ghosts, forming a coherent mass at the bottom of the centrifuge tube. In thirty-six hours L was dead. The peritoneal cavity contained a slimy reddish liquid with numerous cocci, many leucocytes, and some red corpuscles. On centrifugalization, a liquid practically free from hæmoglobin was obtained. Dilute blood-serum was got by rubbing up clot from the heart with sodium chloride solution. The diluted blood-serum caused some laking of rabbit's washed corpuscles, and still more of dog's washed corpuscles. The peritoneal liquid caused considerable laking of rabbit's corpuscles, but not of dog's. The hæmolytic action both of the serum and the peritoneal liquid was abolished by heating to  $59^{\circ}$ . The muscles of the abdominal wall next the parietal peritoneum were altered microscopically and



macroscopically, as in H', although the changes were not so marked. The majority of the fibres showed no cross-striation. The muscles of the thigh were normal.

Two guinea-pigs (M and N) received, intraperitoneally and subcutaneously respectively, washed rabbit's formaldehyde corpuscles, corresponding to 5 c.c. of blood fully fixed. M was dead in less than forty-eight hours. Its peritoneal cavity contained a good deal of a glairy fluid full of leucocytes. No formol-fixed corpuscles could be seen. The blood-serum had no hæmolytic action on rabbit's corpuscles. N was dying, and was killed exactly twenty-four hours after the injection. The blood-serum had some hæmolytic action on washed rabbit's corpuscles, and this was abolished by heating it to 58°. A considerable amount of liquid was found under the skin, with a foul odor and containing numerous bacilli. No trace of formaldehyde-fixed corpuscles could be seen. Many bits of muscular fibres, in which the stripes had sometimes disappeared, were found in the subcutaneous liquid. A kind of rapid digestion of the subcutaneous tissues seemed to have occurred.

Into Guinea-pig P was injected subcutaneously about 1.5 c.c. of a sediment of ghosts obtained by laking rabbit's washed corpuscles with sterile water, and then washing with sterile sodium chloride solution. In forty-two hours it was dead. There was marked subcutaneous œdema, the muscles of the abdominal wall being sodden, dissected-looking, and hemorrhagic. The liquid had a foul odor. The animal contained two well-developed embryos. The amniotic fluid had no hæmolytic action on rabbit's corpuscles suspended in rabbit's serum.

Into another guinea-pig (Q), the washed water-laked ghosts corresponding to 5 c.c. of rabbit's blood were injected subcutaneously. Next day the animal was evidently ill. Forty hours after the injection, it was dying, and blood was obtained from it. There was extensive subcutaneous œdema, the liquid being somewhat turbid and containing hæmoglobin. The muscles of the abdominal wall had the same sodden appearance previously noted, and the microscopic appearances were also similar. The putrid odor of the liquid was evident, although not so marked as on previous occasions, when the autopsy was made some time after death. The blood-serum, and especially the œdema liquid, caused good laking of washed rabbit's corpuscles (abolished by heating to 60°). Marked agglutination, not affected by heating to 60° was also caused, particularly by the

œdema liquid, in which the agglutinated corpuscles seemed to carry down the turbidity and hæmoglobin.

It does not seem possible to attribute all these deaths to faulty technique in the slight operation of injection, particularly as in every case the rapidly fatal result followed the injection of stromata or entire corpuscles; while animals which received hæmoglobin-containing liquid, injected in the same way, survived, although the risk of accidental infection would certainly not be less. The post-mortem appearances showed considerable similarity, and seem to suggest that substances toxic for guinea-pigs may be produced in the stroma of the rabbit's corpuscle by certain laking agents. If this is the case, one of the effects of such toxic substances may be to reduce the defensive power of the tissues against bacterial invasion. In the case of the formaldehyde-fixed corpuscles, it is possible that formaldehyde, liberated from its combination with the stroma or blood-pigment, may have some such effect, since it has been shown by Guthrie<sup>1</sup> to restrain the action of hæmolysins. Where saponin-laked ghosts were injected, the direct toxic influence of the saponin has, of course, to be taken into account.

#### CONCLUSIONS.

1. The stromata and hæmoglobin-containing liquid of colored corpuscles, laked by various agents (for details, see summary, p. 255), cause, when injected into animals of a different species, the production of specific agglutinating and hæmolytic substances. In general, the agglutinating effect is most marked after the injection of stromata, and the hæmolytic effect after the injection of liquid. But the results do not warrant the conclusion that in the intact corpuscle the agglutininogen (the substance which causes the production of agglutinin) is all in the stroma and the hæmolysinogen (the substance which causes the production of hæmolysin), all in the "cellular contents" (in Nolf's sense).

2. Corpuscles fully fixed by formaldehyde cause, on injection, the production of specific agglutinins, and, to a smaller extent, of specific hæmolysins.

3. Corpuscles fully fixed by formaldehyde are capable of being agglutinated by specific sera.

<sup>1</sup> GUTHRIE: This journal, 1903, ix, p. 187.

TABLE I.

Material injected.	Animal.	Agglutination.	Laking.
Saponin-laked ghosts	Rabbit E	Very strong	Fair (less than in Rabbit B)
Intact corpuscles	Rabbit D	Very strong (both for serum and peritoneal fluid)	Strong for serum, stronger than in Rabbit A (little for peritoneal fluid)
Intact corpuscles	G.-p. F	Good	Strong
Ghosts laked by freezing and thawing	G.-p. K	Good	Fair
Liquid after laking by freezing and thawing	G.-p. I	Good	Fair
Heat-laked ghosts	G.-p. J	Good (fair agglutination after 72 days)	Fair (still fair laking after 72 days)
Heat-laked liquid	G.-p. J	Good (some agglutination after 72 days)	Strong (still fair laking after 72 days)
Heat-laked ghosts (with some liquid)	G.-p. B	Good	Good
Ghosts laked by foreign serum	G.-p. A	21	Fair (but less than in G.-p. B)
Water-laked ghosts (excess of water)	Rabbit B	Fair	Fair (better than in rabbit E; about the same as in Rabbit A)
Liquid from water-laked ghosts (laked with excess of water)	Rabbit C	Good	Weak (not abolished by heating to 65° C)
Liquid from water-laked ghosts (laked with minimum of water)	G.-p. O	Good	Fair
Formaldehyde corpuscles (fully fixed)	Rabbit A	Good	Fair (about the same as in Rabbit B)
Formaldehyde corpuscles (imperfectly fixed)	G.-p. H	Good	Strong

Approximate arrangement of the rabbit's sera, as regards agglutinating and hæmolytic power.

*Agglutination.* — E (saponin-laked ghosts), D (intact corpuscles), C (liquid from water-laked corpuscles), A (formaldehyde fixed corpuscles), B (water-laked ghosts).

*Laking.* — D (intact corpuscles), A and B equal (formaldehyde corpuscles and water-laked ghosts), E (saponin ghosts), C (liquid from water-laked). Perhaps C did not live long enough to develop hæmolysin, although long enough for agglutinin.

<sup>1</sup> Observations on agglutination are missing in the notes on this serum.

TABLE II.

*March 10.* — Washed dog's formaldehyde-fixed corpuscles many times with NaCl solution. The dog's blood was obtained on February 15. The corpuscles were then washed and to a suspension of them in NaCl solution, containing about as many corpuscles as the same volume of blood, an equal volume of a 3 per cent solution of formaldehyde in NaCl solution was added. The corpuscles were fully fixed, and the addition of excess of water caused no escape of blood-pigment in several hours. Made a 5 per cent suspension of the fixed corpuscles in NaCl solution (F). Also made a 5 per cent suspension of rabbit's formaldehyde-fixed corpuscles (F'), obtained on February 9 and fixed in the same way as the dog's. Tested the agglutinating power of the serum of rabbits A, B, D, and E, of the serum from the clotted peritoneal fluid of D, and of normal dog's and rabbit's serum on the formaldehyde-fixed corpuscles. In every case 0.5 c.c. of F or F' was used.

- |  |   |
|--|---|
| 1. 0.2 c.c. A serum + F.                 | { The corpuscles sink to the bottom much<br>more quickly than in 3. In few minutes<br>at 40° completely sunk. |
| 2. 0.2 c.c. A serum (heated to 60°) + F. |   |
| 3. 0.2 c.c. NaCl solution + F.           | The corpuscles remain long suspended.   |
| 4. 0.3 c.c. D serum + F.                 | Rapid agglutination or precipitation at 40°.  |
| 5. 0.3 c.c. A serum + F.                 | Rapid agglutination or precipitation, though not so rapid<br>nor so marked as in 4 or 8.                      |
| 6. 0.3 c.c. NaCl solution + F.           | { No agglutination or precipitation at 40°, the cor-<br>puscles remaining long suspended.                     |
| 7. 0.3 c.c. dog's serum + F.             |   |
| 8. 0.3 c.c. peritoneal from D + F.       | Marked agglutination.   |
| 0.3 c.c. D serum + F'.                   | { No agglutination at 40° in 1 hour.  |
| 10. 0.3 c.c. A serum + F'.               |   |
| 11. 0.3 c.c. NaCl solution + F'.         |   |

In the above experiments the agglutination can be seen with the naked eye, and under the microscope the agglutinated specimens show numerous small groups of corpuscles. The agglutination is not difficult to break up by shaking, but under the microscope the clumps can be seen to rapidly form again. The appearances are quite different where salt solution or non-specific sera are added to the formol-fixed corpuscles. It is to be noted that the blood-serum and the serum from the coagulated peritoneal fluid of Rabbit D, which have a stronger agglutinative action than the blood-serum of Rabbit A on dog's fresh corpuscles, have likewise a stronger agglutinative action on dog's formol-fixed corpuscles. No laking of the formol-fixed corpuscles was produced during the period of observation. But after standing at room temperature for 10 days there is laking in all the test-tubes, including the NaCl control test-tubes, the methæmoglobin spectrum being changed into that of oxyhæmoglobin.

TABLE III.

*March 17.* — 10 A. M. : Removed salt solution from the suspensions of dog's and rabbit's formal-fixed corpuscles (F and F') used in experiment of March 16, making the concentration about 10 per cent in each case. In the following observations used always 0.5 c.c. of F or F'. Tested serum of rabbits A, B, D and E, and peritoneal fluid of D.

1. 0.3 c.c. A serum + 0.2 c.c. dog's serum + F. Marked agglutination or precipitation in 5 minutes at room temperature. Kept 1 hour at 40°, and then all night at room temperature. Shook up. The corpuscles sink more rapidly than in (2).
2. 0.3 c.c. A serum + 0.2 c.c. NaCl solution + F. Marked agglutination or precipitation in 5 minutes at room temperature. Kept 1 hour at 40°, then all night at room temperature.
3. 0.3 c.c. D serum + 0.2 c.c. dog's serum + F. Same as (1), but the agglutination is more marked.
4. 0.3 c.c. D serum + 0.2 c.c. NaCl solution + F. Same as (1), but the agglutination is more marked.
5. 0.3 c.c. D serum + F'. No agglutination or precipitation in 5 minutes at 40°. 1 hour at 40°, all night at room temperature. Shook up. Settles far more slowly than (3) or (4).
6. 0.15 c.c. D peritoneal (heated to 58°) + F. Marked agglutination at room temperature in 7 minutes.

*March 18 :*

7. 0.3 c.c. B serum + F. No agglutination in 5 minutes at 40°. Good agglutination in 15 minutes at 40°.
8. 0.3 c.c. E serum + F. Marked agglutination in 5 minutes, even at room temperature.
9. 0.3 c.c. NaCl solution + F. No agglutination in 30 minutes at 40°.
10. 0.3 c.c. E serum + F'. The corpuscles settle somewhat more quickly than in (9), but far more slowly than in (7) or (8).
11. 0.2 c.c. dog's serum (obtained yesterday) + F'. The precipitation is more marked in 20 minutes at 40° than in any of the tubes (12) to (14).
12. 0.2 c.c. normal rabbit's serum + F'. No agglutination or precipitation in 20 minutes at 40°. No agglutination in 12 hours at room temperature.
13. 0.2 c.c. normal rabbit's serum + F. Same result as (12).
14. 0.2 c.c. NaCl solution + F. Same as in (12) and (13).

*March 19 :*

15. 0.3 c.c. E serum (heated to 60°) + F. Marked agglutination in 3 or 4 minutes at room temperature.
16. 0.3 c.c. NaCl solution + F. No agglutination.
17. 0.15 c.c. D peritoneal (heated to 60°) + F. Marked agglutination in 7 minutes at room temperature.
18. 0.15 c.c. A serum (heated to 60°) + F. Good agglutination in 7 minutes, but not so much as (17).
19. 0.3 c.c. B serum (heated to 60°) + F. Rapid and complete precipitation at room temperature.

**Rabbit A.** — 3 kilos. *Feb. 14.* — 5 P. M. : Injected subcutaneously dog's formaldehyde-fixed corpuscles (after thorough washing with NaCl solution) equivalent to 10 c.c. of blood. The corpuscles were obtained by washing fresh dog's blood on February 9. To the washed corpuscles of 60 c.c. of

blood 60 c.c. of a 3 per cent solution of formaldehyde in 0.9 per cent NaCl solution was added. The corpuscles were completely fixed. A little hæmoglobin had escaped during fixation, as is always the case, but none is removed by washing, and the corpuscles are not laked by distilled water even in several hours.

*Feb. 25.* — 4 P. M.: Injected subcutaneously a further quantity of the same washed formaldehyde corpuscles corresponding to 15 c.c. of dog's blood.

*March 15.* — 3 P. M.: Drew some blood from carotid of A, and sewed up wound. Obtained serum from the blood, and tested it on a suspension of dog's washed corpuscles (S) containing 5 per cent of corpuscles, and a dilution of dog's defibrinated blood with NaCl solution (S') containing 10 per cent of blood. 0.5 c.c. of S or S' was always used. The measurements of the liquids were made in long capillary pipettes, the same pipettes being always employed. The liquids were put into very small test-tubes.

1. 0.1 c.c. A serum + S. Good agglutination in few minutes. Slight laking in 30 minutes at 40°, somewhat increased in 16 hours at room temperature, but far less than in (2) or (4).
2. 0.2 c.c. A serum + S. Good agglutination in few minutes. Good laking in 30 minutes at 40°, complete after 16 hours at room temperature.
3. 0.1 c.c. A serum + S'. Good agglutination in few minutes. No laking in 30 minutes at 40°. Very slight laking in 16 hours at room temperature. Less than in (1).
4. 0.2 c.c. A serum + S'. Good agglutination in few minutes. Slight laking in 30 minutes at 40°, increased after 16 hours at room temperature, but much less than in (2).

*March 16.* — 2 P. M.:

- |  |   |   |
|--|---|---|
| <ol style="list-style-type: none"> <li>1. 0.1 c.c. A serum + S. Some laking in 1½ hours at 40°.</li> <li>2. 0.2 c.c. A serum + S. Complete laking in 40 minutes at 40°.</li> <li>3. 0.1 c.c. A serum (heated to 60° for 15 minutes) + S. No laking in 2 hours at 40°.</li> <li>4. 0.2 c.c. A serum (heated to 60° for 15 minutes) + S. No laking in 2 hours at 40°.</li> </ol> | } | Good agglutination in all in few minutes. |
|--|---|---|

*March 16.* — 8 P. M.:

5. 0.2 c.c. A serum (heated to 60°) + 0.1 c.c. dog's serum + S. Some agglutination in 5 minutes at 40°, but much less than in (12) and (13), Rabbit D. Nearly complete and good agglutination in 15 minutes at 40°. Corpuscles are comparatively easily shaken up. No laking after 14 hours at room.
6. 0.2 c.c. NaCl solution + 0.1 c.c. dog's serum + S. No agglutination or laking.
7. 0.3 c.c. dog's serum + 0.2 c.c. NaCl solution + 0.5 c.c. of suspension of A's washed corpuscles. Complete laking in 20 minutes at 40°.
8. 0.3 c.c. dog's serum + 0.2 c.c. A serum + 0.5 c.c. of suspension of A's washed corpuscles. Complete laking in 20 minutes at 40°.
9. 0.5 c.c. NaCl solution + 0.5 c.c. suspension of A's washed corpuscles. No laking in 2 hours at 40°.
10. 0.2 c.c. A serum + 0.5 c.c. of a 5 per cent suspension of normal rabbit's washed corpuscles. No agglutination or laking.

**Rabbit B.** 3 kilos. *Feb. 19.* — 1.45 P. M.: Injected subcutaneously the washed ghosts of 7 c.c. of dog's washed corpuscles after complete laking by water (controlled by microscopic examination), one hour after withdrawal of the blood. Although laking seemed complete, the liquid being perfectly transparent, and the sediment on centrifugalization of a small sample being colorless, the addition of excess of 0.9 per cent NaCl solution caused a heavy precipitate of ghosts, which, on separation by the centrifuge, were seen to be more strongly tinged with hæmoglobin than the supernatant liquid. This sediment was washed twice, each time with 15 times its bulk of NaCl solution. Then water was added to the sediment, so as to be sure that laking was complete.

8 P. M.: Injected subcutaneously into B an additional quantity of washed water-laked ghosts corresponding to 4 c.c. of dog's blood.

*March 1.* — 7 P. M.: Injected subcutaneously the washed water-laked ghosts (corresponding to 20 c.c. of dog's blood) separated from the hæmoglobin-containing liquid injected into Rabbit C.

*March 17.* — 3 P. M.: Got blood from B.

*March 18.* — Made a 5 per cent suspension (S) of dog's fresh washed corpuscles.

1. 0.2 cc. B serum + 0.5 c.c. S. Fair agglutination in 7 minutes at 40°, but not nearly so rapid as in Rabbit E (1). In 20 minutes at 40° good agglutination and fair laking. In 1 hour at 40°, complete laking. Control with NaCl solution. No laking.
2. 0.2 c.c. B serum + 0.5 c.c. of a 5 per cent suspension of normal rabbit's washed corpuscles. No agglutination or laking in 1 hour at 40° and 12 hours at room temperature.

**Rabbit C.** 2 kilos. *March 1.* — 5 P. M.: Injected subcutaneously the hæmoglobin-containing liquid (freed from ghosts) of dog's washed corpuscles, corresponding to 20 c.c. of blood, completely laked by the addition of 55 c.c. of water, as shown by the microscope.

*March 10.* — 2 P. M.: Is dying. There is a growth on the jaw. Got blood, and from this, serum practically free from hæmoglobin.

*March 11.* — 2 P. M.: Got blood from a dog. Made a suspension containing 5 per cent of dog's washed corpuscles (S), and a dilution of the dog's blood with NaCl solution, containing 10 per cent of blood (S'). 0.5 c.c. of S or S' was always used.

1. 0.1 c.c. C serum + S. Little if any laking at 40° in 1 hour. Some laking after 2 hours at 40° and 14 hours at room temperature, but quite incomplete. Good agglutination, coming on almost at once.
2. 0.2 c.c. C serum + S. Little if any laking at 40° in 1 hour. Some laking in 2 hours at 40°, not much increased in 14 hours at room temperature. Agglutination quite marked, coming on almost at once.

3. 0.4 c.c. C serum + S. No laking in 30 minutes at 40°, and little in 14 hours at room temperature. After 1 hour more at 40°, and shaking up sediment, decidedly more laking than in (6).
4. 0.1 c.c. C serum + S'. No laking at 40° in 2 hours. Good agglutination. Practically no laking in 14 hours more at room temperature.
5. 0.2 c.c. C serum + S'. No laking at 40° in 2 hours. Good agglutination. Slight laking in 14 hours more at room temperature, but less than in (2).
6. 0.4 c.c. C serum + S'. No laking in 30 minutes at 40°, and very little in 14 hours at room temperature. Somewhat more after shaking up sediment and 1 hour more at 40°.
7. 0.2 c.c. C serum (heated to 60°) + S. No laking at 40° in 30 minutes. Good agglutination. Still good agglutination after 14 hours at room temperature, and slight laking. After 1 hour more at 40°, laking is as good as in (2).
8. 0.2 c.c. C serum (heated to 60°) + S'. No laking at 40° in 30 minutes. No agglutination after 14 hours at room temperature and another hour at 40°, still no agglutination. Slight laking, but decidedly less than in (7).
9. 0.2 c.c. NaCl solution + S'. No laking after 2 hours at 40° and 14 hours at room temperature, except a slight ring above sediment.

In all this experiment the laking is inconspicuous and not abolished by heating the serum. The agglutination is the striking feature.

**Rabbit D.** 2 kilos. *March 1.* — 11.30 A.M.: Injected subcutaneously washed dog's corpuscles corresponding to 27 c.c. of blood.

*March 15.* — 2 P.M.: Looks ill and emaciated. Salivating considerably. Swelling on right upper jaw. An abscess under skin of abdomen containing inspissated pus. No injection was made in this situation. Got blood-serum free from hæmoglobin. Much clear liquid in peritoneal cavity which clots rapidly on withdrawal. Obtained serum from this clot, and tested its hæmolytic and agglutinating power. Used suspension of dog's washed corpuscles containing 5 per cent of corpuscles (S). Also dilution of dog's blood containing 10 per cent of blood (S'). 0.5 c.c. of S or S' was always used.

1. 0.1 c.c. D serum + S. Complete agglutination, although not so rapidly as in (7). Marked laking in 20 minutes at 40°.
2. 0.2 c.c. D serum + S. Complete agglutination in 1 or 2 minutes. Marked laking in 20 minutes at 40°.
3. 0.1 c.c. D serum + S'. Same as (1) and (2).
4. 0.2 c.c. D serum + S'. Same as (1) and (2).
5. 0.2 c.c. D serum (heated to 60°) + S'. Marked agglutination. No laking in 30 minutes at 40°, but slight laking after 1 hour more at 40° and 1 hour at room temperature.
6. 0.2 c.c. D serum (heated to 60°) + S. Marked agglutination. No laking at 40° in 40 minutes. Slight laking in 2 hours at 40° and 1 hour at room temperature, though distinctly less than in (5).
7. 0.1 c.c. peritoneal + S. Complete and rapid agglutination as in (1). No laking in 2 hours at 40°.
8. 0.2 c.c. peritoneal + S. Complete and rapid agglutination as in (1). No laking in 2 hours at 40°.



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9. 0.1 c.c. peritoneal + S'. Marked agglutination. Very little laking in 2 hours at 40° and 1 hour, room temperature.
10. 0.2 c.c. peritoneal + S'. Marked agglutination. Very little laking in 2 hours at 40° and 1 hour, room temperature, but somewhat more than in (9), though far less than in (3) and (4).
11. 0.2 c.c. peritoneal (heated to 60°) + S'. Marked agglutination. No laking in 2 hours at 40°, and 1 hour at room temperature.

*March 16.* — 8 P. M. :

12. 0.2 c.c. D serum (heated to 60°) + 0.1 c.c. dog's serum + S. Complete agglutination in 5 minutes at 40°, more rapid than in (13) or in Rabbit A (5). After 14 hours at room temperature, slight laking. The corpuscles form a solid mass, not easily shaken up, at bottom of tube.
13. 0.2 c.c. peritoneal (heated to 60°) + 0.1 c.c. dog's serum + S. Almost complete agglutination in 5 minutes at 40°, complete in 10 minutes at 40°. After 14 hours the corpuscles form a solid mass, not easily shaken up. Slight laking, more than in (12).
14. 0.2 c.c. NaCl solution + 0.1 c.c. dog's serum + S. No agglutination or laking.

*March 18.* — 5 P. M. :

15. 0.2 c.c. D serum + 0.5 c.c. of a 5 per cent suspension of normal rabbit's washed corpuscles. No agglutination or laking in 1 hour at 40° and 12 hours, at room temperature.
16. 0.2 c.c. peritoneal + 0.5 c.c. of the suspension of rabbit's corpuscles. No laking in 1 hour at 40° and 12 hours at room temperature. Possibly slight agglutination. It is more difficult to shake up into complete suspension than (15), but does not settle more rapidly.

**Rabbit E.** 2 kilos. *March 7.* — 8 P. M. : Injected subcutaneously the twice-washed ghosts of dog's washed corpuscles (corresponding to 25 c.c. of blood) after laking with 2.5 c.c. of a 3 per cent solution of saponin in 0.9 per cent NaCl solution. The saponin solution was boiled before being added. It did not produce rapid laking, and the quantity added was probably not far from the minimal dose for complete laking.

*March 8.* — 11 A. M. : Injected subcutaneously the washed saponin-laked ghosts corresponding to 5 c.c. more of the dog's blood.

*March 17.* — 3 P. M. : Got blood from E, and allowed to clot. Kept in ice-chest.

*March 18.* — Made a 5 per cent suspension of dog's fresh washed corpuscles (S). Obtained serum from the blood of E.

1. 0.2 c.c. E serum + 0.5 c.c. S. Almost immediate complete agglutination. Shook up. In 20 minutes at 40° some laking, but less than in Rabbit B (1). In 1 hour at 40°, almost complete laking.
2. 0.2 c.c. NaCl solution + 0.5 c.c. S. No laking in 1 hour at 40°.
3. 0.2 c.c. E serum + 0.5 c.c. of a 5 per cent suspension of normal rabbit's washed corpuscles. No agglutination or laking in 1 hour at 40° and 12 hours at room temperature.

**Guinea-pig A.** 750 grams. *Jan. 18.* — To 25 c.c. of a suspension of washed rabbit's corpuscles corresponding to 6.5 c.c. of blood added 15 c.c. of dog's serum. Placed the mixture at 40° for 25 minutes. Laking

complete. Washed the ghosts repeatedly with NaCl solution, and injected them intraperitoneally into A.

*Feb. 11.* — 11 A.M. : Got defibrinated blood, and separated the serum at once by centrifuge. Tested at 2 P.M. on a suspension of washed rabbit's corpuscles containing about 25 per cent of corpuscles, and, on a suspension of dog's washed corpuscles of the same strength.

- 1 1 c.c. A serum + 1 c.c. rabbit's suspension. Good laking at 40° in less than 1 hour.
- 2 1 c.c. A serum (heated to 58°) + rabbit's suspension. No laking after 1 hour at 40° and 20 hours at 0°.
- 3 1 c.c. A serum + 1 c.c. dog's suspension. No laking in 2 hours at 40°. Slight laking after 20 hours at 0°.
- 4 Dog's serum + A's blood (serum and corpuscles). Complete laking at 40° in 1 hour.
- 5 Dog's serum + rabbit's suspension. Fair laking at 40° in 1 hour, but not nearly as much as in (4).
- 6 Dog's serum + A's washed corpuscles. Complete laking in a few minutes at 40°.
- 7 Dog's serum + excess of A's serum + A's washed corpuscles. Complete laking in few minutes at 40°.
- 8 Normal guinea-pig's serum + A's washed corpuscles. No laking.

*February 13 :*

- 9 A serum (kept at 0° since Feb. 11) + rabbit's washed corpuscles + an equal volume of rabbit's serum. Practically no laking in 1 hour at 40° and 16 hours at room temperature.
- 10 A serum - rabbit's washed corpuscles. Some laking, but the laking power of A serum is now feeble compared with (1), although distinctly greater than in (9).

**Guinea-pig B.** *Jan. 18.* — Laked a portion of the same suspension of rabbit's washed corpuscles as was used for Guinea-pig A, by heating to 55° C. for 40 minutes and then to 61° for 4 minutes. Even about 55° to 58° a brownish precipitate came down. Centrifugalized off a portion of the hæmoglobin-containing liquid, and injected the sediment, with the balance of the liquid, into B intraperitoneally. The amount injected contained the ghosts of 5 c.c. of blood. Another specimen of the suspension of rabbit's corpuscles was heated to 58° for 15 minutes. The same brownish precipitate came down. The same was the case for specimens heated to 61° and to 64°. The precipitate was very copious, and under the microscope was seen to consist of ghosts covered with numerous blackish granules, suggesting hæmatin, many of them free. All the corpuscles seemed to have lost their hæmoglobin. With the most cautious heating which would cause laking it was found impossible to avoid this precipitate in suspensions of washed corpuscles. It is quite different when entire defibrinated blood is laked by heat, possibly because the alkaline reaction of the latter prevents the heat-coagulation of the pigment even at 62° to 63°. On heating some of the hæmoglobin-containing liquid to 61° a fair precipitate came down.

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*Feb. 12.* — Obtained defibrinated blood, and immediately centrifugalized off the serum. Used the same suspension of rabbit's washed corpuscles (S) as was used for testing Guinea-pig A's serum.

1. B serum + S. Complete laking in 30 minutes at 40°.
2. B serum (heated to 58°) + S. No laking in 2 hours at 40°.
3. Serum of normal guinea-pig + S. Some laking in 1½ hours at 40°, but much less than in (1).
4. Serum of normal guinea-pig (heated to 58°) + S. No laking in 1 hour at 40°, and 16 hours at room temperature.
5. Serum of normal guinea-pig + S + equal volume of rabbit's serum. Practically no laking in 1½ hours at 40°. In a second experiment no laking whatever in 1 hour at 40° and 16 hours at room temperature.
6. Repetition of (5) except that the serum of normal guinea-pig was heated to 58°. No laking.
7. B serum + S + equal volume of rabbit's serum. Good laking in 1½ hours at 40°. In a second experiment, practically complete laking in 1 hour at 40° and 16 hours at room temperature.
8. Rabbit's serum + B's blood + excess of B's serum. Complete laking at 40° in 40 minutes.

*February 14.* — 5.30 P. M. :

9. B serum (heated to 59°) + equal volume rabbit's serum + 3 volumes S. Good agglutination in 15 minutes at 40°. No laking in 40 minutes.
10. 1 volume B serum + 1 volume rabbit's serum + 3 volumes S. Fair laking in 15 minutes at 40°. Good agglutination. After 40 minutes at 40°, the laking is still not so great as in (1), but distinct.
11. 1 volume B serum + 1 volume 0.9 per cent NaCl solution + 3 volumes S. Good laking and agglutination in 15 minutes at 40°. After standing 14 hours at room temperature, no alteration in (9), (10), or (11).
12. 1 volume of another normal guinea-pig's serum + 3 volumes S. Fair laking after 15 minutes at 40°. Also agglutination, the corpuscles being mostly round and swollen. Laking not increased after 2 hours at 40°, and 18 hours at room temperature. Repeated (12) with normal guinea-pig's serum heated to 58°. No laking or agglutination. Corpuscles mostly round, not crenated.
13. 1 volume normal guinea-pig's serum + 1 volume rabbit's serum + 3 volumes S. No laking or agglutination in 2 hours at 40°, and 18 hours at room temperature. The corpuscles are much crenated in general.

**Guinea-pig F.** 500 grams. *Jan. 20.* — Injected intraperitoneally rabbit's corpuscles (corresponding to 3 c.c. of blood) washed with NaCl solution.

*March 2.* — Obtained blood from carotid. Sewed up wound. In the following experiments used a suspension of rabbit's washed corpuscles (S), and a dilution of rabbit's blood (S'), each containing 5 per cent of corpuscles.

1. 0.1 c.c. F serum + S. Complete laking in 15 minutes at 40°.
2. 0.1 c.c. F serum + S'. Complete laking in 30 minutes at 40°.

*March 7:*

3. 0.2 c.c. dog's serum + 0.5 c.c. of a 5 per cent suspension of F's washed corpuscles. Complete laking in 30 minutes at 40°.
4. 0.2 c.c. rabbit's serum + 0.5 c.c. of a 5 per cent suspension of F's washed corpuscles. Slight laking at 40° in 30 minutes. Still incomplete in 2½ hours at 40°.
5. 0.2 c.c. F serum + 0.5 c.c. of a 5 per cent suspension of dog's washed corpuscles. No laking at 40° in 1½ hours. Slight laking in 3 hours at 40°.
6. 0.2 c.c. dog's serum + 0.5 c.c. of a 10 per cent dilution of F's blood. Complete laking at 40° in 10 minutes.
7. 0.2 c.c. rabbit's serum + 0.5 c.c. of a 10 per cent dilution of F's blood. Very little laking in 10 minutes. Fair laking in 2 hours at 40°.
8. 0.2 c.c. F serum + 0.2 c.c. rabbit's serum + 0.5 c.c. of a 10 per cent suspension of rabbit's washed corpuscles. Good agglutination and fair laking in 15 minutes at 40°. Good laking after 1 hour more at 40° and 16 hours at room temperature.
9. 0.2 c.c. F serum + 0.2 c.c. dog's serum + 0.5 c.c. of the same suspension of dog's corpuscles as was used in K (19). No laking at 40° in 1½ hours. Slight laking in 3 hours at 40°, but less than in K (19). After 16 hours at room temperature, laking not marked.
10. 0.2 c.c. F serum (heated to 58°) + 0.2 c.c. rabbit's serum + 0.5 c.c. of 10 per cent suspension of rabbit's washed corpuscles. No laking in 40 minutes at 40°, but good agglutination. No laking in 2 hours at 40° and 16 hours at room temperature.

**Guinea-pig G.** *Jan. 23, 1904.* — 3 c.c. of dog's blood kept in sealed tubes at room temperature since Feb. 18, 1901, when it was drawn under aseptic conditions, was injected intraperitoneally into G.

*Jan. 24, 1904.* — Injected subcutaneously into G some of the sediment after washing with NaCl solution, and also some of the hæmoglobin-containing liquid. The blood was obtained from the dog's carotid by pushing the points of sterile pipettes through the vessel wall and then breaking them off. The points were sealed in the flame. The clot had not entirely disappeared. It was broken up by rubbing in NaCl solution, and portions of the suspension and of the hæmoglobin-containing liquid were injected. The spectrum before breaking the tubes was observed. The bands of oxyhæmoglobin were well marked. There was no band in the red in the position of the methæmoglobin or acid hæmatin band. The reaction was about neutral to blue and red litmus paper. The blood was well laked. The spectrum was not altered by opening the tubes and diluting a specimen with water. Bouillon tubes inoculated with the blood showed no growth.

Microscopically, some ghosts of normal size were seen, and numerous smaller colorless bodies, often irregular in shape, perhaps shrunken ghosts or portions of such; numerous clumps of blood-pigment granules, not containing any intact corpuscles; many beautiful tyrosin crystals in sheaves, half-sheaves, and isolated needles, soluble only in large excess

of water, insoluble in alcohol; also some cholesterin crystals. Some reddish crystals are also present, in the form of tables, perhaps reduced hæmoglobin. If oxyhæmoglobin, they are atypical in form. They are not hæmatoidin, because easily soluble in water and insoluble in chloroform, and they do not give Gmelin's reaction. Chloroform takes up none of the red pigment in this long-kept blood. Some yellowish-red balls are present which are soluble in water, and suggest leucin, but are insoluble in alcohol. Even boiling alcohol does not seem to take up anything from the blood. All the pigment in the sediment, after washing it with NaCl solution, is not soluble in water. A considerable brownish residue remains which is soluble in dilute potassium hydrate, but practically insoluble in dilute mineral acid (nitric acid). The insoluble pigment when pressed between two glass slides shows no distinct spectrum. On the addition of ammonium sulphide, a strong hæmochromogen spectrum is obtained. Dissolved in water some of the sediment containing the reddish crystals. The red liquid gives a faint spectrum, two poorly defined bands in the position of the oxyhæmoglobin bands. Boiled the solution. The pigment is not all precipitated by boiling, and therefore the filtrate shows the same spectrum. The addition of ammonium sulphide to the filtrate gives good hæmochromogen bands.

For the original fresh dog's defibrinated blood, on Feb. 18, 1901, the conductivity (at 5° C.)  $\times 10^8$  was 34.12; for the hæmoglobin-containing liquid on Jan. 23, 1904, it was 92.85; and for the mixture of liquid and sediment, 75.22, expressed in reciprocal ohms.

The point of the pipette in which one of the samples of the dog's blood was taken got broken in transportation two years after the blood was drawn. It was found sealed up by some dried blood. The contents had no putrefactive odor, but a musty smell. No tyrosin crystals were present. The clot had not entirely disappeared. All the corpuscles were laked, and intact ghosts were not to be found. Bouillon tubes inoculated from this pipette immediately after it was opened showed a good growth containing numerous micrococci and a smaller number of a motile bacillus.

*Jan. 28.* — G is dead. Got clotted blood from the right heart. The left heart is empty. Broke up the clot in NaCl solution, and centrifugalized. Obtained from thoracic cavity some liquid containing red corpuscles. Centrifugalized. The supernatant liquid is nearly free from hæmoglobin.

1. 1 c.c. of a suspension of dog's washed corpuscles + 1 c.c. of G's dilute blood-serum. No laking after 2 hours at 40°.
2. 1 c.c. of dog's suspension + 1 c.c. of G's dilute blood-serum (after heating 20 minutes to 59°). No laking in 2 hours at 40°.
3. 1 c.c. of dog's suspension + 1 c.c. thoracic liquid from G. Good laking in 2 hours. at 40°.

4. 1 c.c. of a suspension of rabbit's washed corpuscles (corresponding to 0.1 c.c. blood) + 1 c.c. of G's thoracic liquid. Complete laking in  $1\frac{1}{2}$  hours at  $40^{\circ}$ .
5. 1 c.c. of rabbit's suspension + 1 c.c. NaCl solution. No laking after  $1\frac{1}{2}$  hours at  $40^{\circ}$  and 14 hours at  $0^{\circ}$ .
6. 1 c.c. of rabbit's suspension + 1 c.c. G's diluted blood-serum. No laking after  $1\frac{1}{2}$  hours at  $40^{\circ}$  and 14 hours at  $0^{\circ}$ .
7. 1 c.c. of rabbit's suspension + 1 c.c. G's thoracic liquid (after heating to  $59^{\circ}$ ). No laking after  $1\frac{1}{2}$  hours at  $40^{\circ}$  and 14 hours at  $0^{\circ}$ .

**Guinea-pig H.** *Jan. 21.* — 8.30 P.M.: Injected washed rabbit's corpuscles imperfectly fixed by formaldehyde, and then thoroughly washed with NaCl solution. A week ago a suspension of rabbit's washed corpuscles, containing the same proportion of corpuscles as the original blood, was made. To this was added half its volume of a 3 per cent solution of formaldehyde in NaCl solution.

The fixing was not so complete but that water caused complete laking. But there was no putrefaction. A little hæmoglobin was present in the NaCl solution after each washing, even the last, and it did not perceptibly diminish in the last few washings, indicating that the NaCl solution was producing slight laking of the corpuscles. (I have previously shown that NaCl penetrates more easily than normal into corpuscles in the first stage of fixing by formaldehyde.)

*Feb. 16.* — 11.30 A.M.: Got blood from carotid. Sewed up wound. Made a dilution of rabbit's blood containing 5 per cent of corpuscles. To two samples of this added, respectively, H's serum and serum from a normal guinea-pig in the same amount. Complete laking in the tube with H's serum in 10 minutes at  $40^{\circ}$ . Good agglutination. No laking in the other. After 2 hours at  $40^{\circ}$  and 16 hours at room temperature, slight laking in tube with normal serum.

H's serum + washed rabbit's corpuscles (*a*). Fair laking after 2 hours at  $40^{\circ}$  and 16 hours at room temperature.

Normal guinea-pig's serum + washed rabbit's corpuscles (*b*). Little laking after 2 hours at  $40^{\circ}$  and 16 hours at room temperature.

After 1 hour at  $40^{\circ}$  (during which there was some laking in tube *a*, none in *b*) added to a portion of *a* and of *b* excess of rabbit's serum. After 1 hour more at  $40^{\circ}$ , and 16 hours at room temperature, laking was about the same in *a* + rabbit's serum, as in *a* at end of same period. In *b* + rabbit's serum, the laking was even less than in *b*.

**Guinea-pig I.** *Jan. 27.* — Injected intraperitoneally the hæmoglobin-containing liquid of 15 c.c. of a suspension of washed rabbit's corpuscles (corresponding to 7.5 c.c. of blood) after complete laking by freezing and thawing 4 times, and prolonged centrifugalization.

*Feb. 7.* — Ill, refuses food.

*Feb. 8.* — Killed at 9 P.M. Got serum from defibrinated blood. No pathological appearances under the skin or in the peritoneal cavity.

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*Feb. 9.* — I serum + suspension of rabbit's corpuscles. Complete laking.

I serum (heated to  $57^{\circ}$ ) + suspension of rabbit's corpuscles. No laking in 3 hours at  $40^{\circ}$  and 17 hours at room, but strong agglutination.

Normal rabbit's serum + washed corpuscles of I. Good laking at  $40^{\circ}$  in  $2\frac{1}{2}$  hours.

Normal rabbit's serum + unwashed corpuscles of I. Fair laking at  $40^{\circ}$  in  $2\frac{1}{2}$  hours.

I serum + washed corpuscles of N. Little, if any, laking in  $2\frac{1}{2}$  hours at  $40^{\circ}$ . A little laking in 16 hours at room temperature.

**Guinea-pig J.** 600 grams. *Jan. 26.* — Injected subcutaneously the hæmoglobin-containing liquid from washed rabbit's corpuscles (corresponding to 7 c.c. of defibrinated blood) after laking by heating to  $60^{\circ}$  for 15 minutes, and prolonged centrifugalization.

*Feb. 16.* — Obtained some blood from the ear of J. Shook up with some NaCl solution and centrifugalized. Added the clear liquid to a dilution of rabbit's blood and to a suspension of washed rabbit's corpuscles. Very little laking in either after 2 hours at  $40^{\circ}$  and 16 hours at room temperature. Good agglutination in both.

*Feb. 18.* — Got blood from carotid of J. Ligated vessel and sewed up wound.

J serum + rabbit's blood dilution. Complete laking in 15 minutes at  $40^{\circ}$ . Good agglutination.

J serum + rabbit's washed corpuscles. Marked laking in 15 minutes at  $40^{\circ}$ . Good agglutination.

*April 7.* — Got blood from carotid of J. Sewed up wound. Made a 5 per cent suspension of washed rabbit's corpuscles (S), and a 10 per cent dilution of rabbit's blood (S'). Used always 0.5 c.c. of S and S'.

1. 0.1 c.c. J serum + S. Slight laking in 30 minutes at  $40^{\circ}$ . Fair laking in 45 minutes at  $40^{\circ}$ . After 12 hours at room temperature, laking still incomplete.
2. 0.2 c.c. J serum + S. Good laking in 30 minutes at  $40^{\circ}$ , but not complete. Then 15 minutes more at  $40^{\circ}$ . After 12 hours at room temperature, practically complete laking.
3. 0.2 c.c. J serum (heated to  $60^{\circ}$ ) + S. No agglutination to eye in 45 minutes at  $40^{\circ}$  and 12 hours at room temperature.
4. 0.2 c.c. NaCl solution + S. No laking or agglutination.

Repeated (1) to (4) with S' instead of S. Same result, except that in tube corresponding to (2) laking was not quite complete.

Microscopically, J serum causes fair agglutination of S and S' in the proportion of 2 of the serum to 5 of S or S'.

The same is true of J serum after heating to  $62^{\circ}$  for 7 minutes. The agglutination is less than with J' serum.

*April 8.* — Laked 3.5 c.c. of rabbit's blood by heating to  $62^{\circ}$  for 15 minutes. Centrifugalized off the ghosts thoroughly, as shown by the microscope. Injected the liquid subcutaneously into J.

*April 28.* — 10 A.M. Obtained blood from J. Used the same suspension of rabbit's corpuscles (S) and the same dilution of rabbit's blood (S') as were used in testing the serum of J'. Always 0.5 c.c. of S or S'.

1. 0.1 c.c. J serum + S. Some laking in 8 minutes at  $40^{\circ}$ , but distinctly less than in corresponding tube with J' serum. Good agglutination. Nearly complete laking in 20 minutes at  $40^{\circ}$ . Complete in 40 minutes at  $40^{\circ}$ , but a greater sediment of ghosts than in J'.
2. 0.1 c.c. J serum (heated 20 minutes to  $60^{\circ}$ ) + S. Even after  $1\frac{1}{2}$  hours there is little if any, agglutination. No laking.
3. 0.05 c.c. J serum + S. Some laking in 5 minutes at  $40^{\circ}$ , but less than in the corresponding J' tube. Laking still incomplete in 25 minutes at  $40^{\circ}$ . Complete in 40 minutes at  $40^{\circ}$ , and one hour at room temperature.
4. 0.05 c.c. J serum + S. At room temperature, for 12 hours. Strong agglutination, all the corpuscles being in a mass, which is not easily shaken up. No laking.

*April 29.* — 10 A.M.:

5. 0.1 c.c. J serum + S'. Complete laking at  $40^{\circ}$  in 9 minutes.
6. 0.1 c.c. J serum (heated to  $56^{\circ}$  for 18 minutes) + S'. Good agglutination in 20 minutes at  $40^{\circ}$ . Shook up and left at room temperature for 30 minutes. Some laking. None in control tube with NaCl.
7. 0.1 c.c. J serum (heated to  $60^{\circ}$  for 20 minutes) + S'. After 10 minutes at  $40^{\circ}$ , and 4 hours at room temperature, some agglutination, and slight laking. None in control. Microscopically, both J and J' serum, heated to  $56^{\circ}$  for 12 minutes, cause marked agglutination of washed rabbit's corpuscles, much better marked than in the case of same sera after being heated to  $60^{\circ}$ . This suggests that the agglutinin formed after the injection of stroma, or liquid which has been heated to  $60^{\circ}$ - $62^{\circ}$ , is perhaps rendered inactive at about the same temperature, though not at  $56^{\circ}$ , while agglutinin produced under the influence of stroma, or liquid which has not been heated, is not rendered inactive by heating it to  $60^{\circ}$ .

Mixed J serum and a sediment of rabbit's washed corpuscles, both previously cooled to  $0^{\circ}$ , and allowed the mixture to stand in ice for  $2\frac{3}{4}$  hours. Then centrifugalized off the serum. Call the serum B. It is somewhat tinged with hæmoglobin.

8. 0.1 c.c. B + S. Little, if any, laking or agglutination in 30 minutes at  $40^{\circ}$ .
9. 0.1 c.c. J serum (heated to  $56^{\circ}$ ) + 0.05 c.c. B + S. Fair laking and good agglutination in 30 minutes at  $40^{\circ}$ . After 40 minutes more, at  $40^{\circ}$ , marked laking. Much more than in tube 8, though not yet so much as in 10.
10. 0.1 c.c. J serum + 0.05 c.c. NaCl solution + S. Good laking and agglutination in 10 minutes at  $40^{\circ}$ . Washed the sediment of corpuscles with which B was in contact, and made a 5 per cent suspension of it in NaCl solution.
11. 0.5 c.c. of this suspension + 0.1 c.c. B. Some laking in 1 hour at  $37^{\circ}$ , and marked agglutination. In 30 minutes more, fair laking. In 16 hours, at room temperature, good laking. Some agglutination, but no laking, in a control tube with NaCl solution instead of B.
12. 0.5 c.c. of the suspension + 0.1 c.c. B (heated to  $56^{\circ}$ ). No laking.



**Guinea-pig J'.** 850 grams. *Jan. 26.* — Injected intraperitoneally the washed sediment of a suspension of washed rabbit's corpuscles (corresponding to 7 c.c. of defibrinated blood) laked by heating to 60° for 15 minutes. The hæmoglobin-containing liquid was injected into J. The sediment was washed repeatedly with NaCl solution.

*Feb. 17.* — Got blood from carotid. Sewed up wound. Used 10 per cent dilution of rabbit's blood (S').

J' serum + S'. Good laking at 40° in 1 hour.

J' serum (heated to 58°) + S'. Good agglutination. No laking in 2 hours at 40° and 16 hours at room temperature.

Serum of normal guinea-pig + S'. No laking in 2 hours at 40° and 16 hours at room temperature.

*April 7.* — Got blood from carotid. Sewed up wound. Used 5 per cent suspension of washed rabbit's corpuscles (S) and 10 per cent dilution of rabbit's blood (S') (always 0.5 c.c.).

1. 0.1 c.c. J' serum + S'. Little, if any, laking in 30 minutes at 40°. In 3 hours at 40° considerable laking.
2. 0.2 c.c. J' serum + S'. Complete laking in 15 to 20 minutes at 40°.
3. 0.2 c.c. J' serum (heated to 60°) + S'. No laking. No agglutination (to eye) in 30 minutes at 40°. Fair agglutination (to eye) in 3 hours at 40°.

(1) to (3) were repeated with S, with similar results. Also repeated with a 10 per cent dilution of dog's blood instead of S and S', with a negative result as regards both laking and agglutination.

Microscopically, J' serum caused good agglutination of S and S' when added in the proportion of 2 of the serum to 5 of S or S'.

*April 8.* — Injected subcutaneously into J' the thoroughly washed heat-laked ghosts of 3.5 c.c. of rabbit's blood (whose liquid was injected into J).

*April 28.* — 10 A.M. Obtained blood from external jugular. Sewed up wound. Made a 5 per cent suspension of washed rabbit's corpuscles (S), and a 10 per cent dilution of rabbit's blood (S'). Used always 0.5 c.c. of S or S'.

1. 0.1 c.c. J' serum + S. Complete laking in 8 minutes at 40°.
2. 0.1 c.c. J' serum (heated 20 minutes to 60°) + S. Little, if any, agglutination. No laking.
3. 0.05 c.c. J' serum + S. Fair laking in 5 minutes at 40°, and complete laking in 20 minutes at 40°.
4. 0.05 c.c. J' serum + S. At room temperature for 12 hours. Strong agglutination. No laking. Same as in corresponding J tube. The contrast between J (4) and J' (4), on the one hand, and J (2) and J' (2), on the other, is great. In the latter two tubes the sediment, after standing 15 hours at room temperature, shakes up about as readily as in the control with NaCl solution.

*April 29.* — 10 A.M. :

5. 0.1 c.c. J' serum + S'. Complete laking at 40° in 9 minutes.

6. 0.1 c.c. J' serum (heated to 56° for 18 minutes) + S'. Good agglutination in 20 minutes at 40°; the precipitation of the corpuscles is more complete than in the corresponding J tube. Shook up and left 30 minutes at room temperature. Some laking. None in control tube with NaCl.

**Guinea-pig K.** — 800 grams. *Jan. 27.* — The sediment of ghosts (corresponding to 7.5 c.c. of rabbit's blood, laked by freezing and thawing), whose extracorporeal liquid was injected into Guinea-pig I, was washed with NaCl solution and injected intraperitoneally into K.

*March 2.* — Obtained blood from carotid. The animal contained two embryos, from which a little blood was got, also some amniotic and allantoic fluids. Used the same suspension of washed rabbit's corpuscles (S), and the same dilution of rabbit's blood (S'), each containing 5 per cent of corpuscles, as was employed in testing the serum of the control Guinea-pig F. 0.5 c.c. of S or S' was always used.

1. 0.1 c.c. K serum + S. Good laking (not complete) in 1 hour at 40°. Still incomplete after 2½ hours at 40° and 15 hours at room temperature. With F serum, complete in 15 minutes at 40°. See F (1).
2. 0.1 c.c. K serum + S'. Very slight laking in 2 hours at 40° and 15 hours at room temperature. With F serum, complete in 30 minutes at 40°. See F (2).
3. 0.25 c.c. K serum + S'. Marked laking in 10 minutes at 40°, and good agglutination.
4. 0.25 c.c. amniotic liquid + S. } No laking or agglutination in 2½ hours at 40°.
5. 0.25 c.c. allantoic liquid + S. } Same for S'.
6. 0.2 c.c. K serum (heated to 58°) + S. No laking in 2 hours at 40°. Good agglutination.
7. 0.2 c.c. K serum + S. Complete laking in 30 minutes at 40°.
8. 0.1 c.c. embryo's serum + S. No laking in 10 minutes at 40°.
9. 0.2 c.c. embryo's serum + S. } No laking in 2 hours at 40° and 15 hours at room tem-
10. 0.3 c.c. embryo's serum + S. } perature.
11. 0.2 c.c. K serum + S'. Fair laking in 2 hours at 40° and 15 hours at room temperature, but far less than in (7).
12. 0.2 c.c. K serum (heated to 60°) + 0.2 c.c. amniotic liquid + S. } No laking in 3 hours at 40°. (No
13. 0.2 c.c. K serum (heated to 60°) + 0.2 c.c. allantoic liquid + S. } complement in the liquids.)

K's blood + normal rabbit's serum. Marked laking at 40° in 30 minutes.

Embryo's blood + normal rabbit's serum. No laking at 40° in 30 minutes, nor in 1½ hours at 40°, but slight laking in 15 hours at room temperature, while control with NaCl solution shows no laking.

#### *March 4:*

14. 0.2 c.c. K serum + 0.5 c.c. of a 5 per cent suspension of dog's washed corpuscles. No laking at 40° in 1½ hours. Slight laking in 3 hours at 40°.
15. 0.2 c.c. dog's serum + 0.5 c.c. of a 5 per cent dilution of K's blood. Complete laking at 40° in 10 minutes.
16. 0.2 c.c. rabbit's serum + 0.5 c.c. of a 5 per cent dilution of K's blood. Very little laking in 10 minutes. Fair in 2 hours at 40°.
17. 0.2 c.c. rabbit's serum + 0.5 c.c. of a 5 per cent suspension of washed K's corpuscles. No laking in 10 minutes, good laking in 2 hours at 40°.

18. 0.2 c.c. dog's serum + 0.5 c.c. of a 5 per cent suspension of washed K's corpuscles. Complete laking in 10 minutes at  $40^{\circ}$ .
19. 0.2 c.c. K serum + 0.2 c.c. dog's serum + 0.5 c.c. of a 5 per cent suspension of dog's washed corpuscles (the same as was used in F (5) and F (9)). No laking at  $40^{\circ}$  in  $1\frac{1}{2}$  hours. Some laking at  $40^{\circ}$  in 3 hours, but even after 16 hours at room temperature laking is not marked.
- The rabbit's serum was the same as that used in F (+), (7), (8), and (10).

**Guinea-pig O.** *Feb. 9.* — Injected partly intraperitoneally and partly subcutaneously the hæmoglobin-containing liquid from rabbit's washed corpuscles (corresponding to 8 c.c. of blood) laked with a minimum of distilled water and centrifugalized for a long time.

*Feb. 18.* — Has not been looking well for 3 days. A considerable abscess was found on the back, not in the position of the injection puncture. Opened and washed with corrosive sublimate and then boiled water.

*Feb. 19.* — Is dying. Got blood and separated the serum at once. No pathological lesion except the abscess could be found. Used a 10 per cent suspension of rabbit's defibrinated blood (unwashed).

O's serum + suspension. Very fair laking in  $1\frac{1}{2}$  hours at  $40^{\circ}$ , not quite complete in 16 hours at room temperature.

O's serum (heated to  $58^{\circ}$ ) + suspension. Good agglutination. No laking.

Normal guinea-pig's serum + suspension. No laking in  $1\frac{1}{2}$  hours at  $40^{\circ}$  and 16 hours at room temperature.

*Feb. 24.* — 0.15 c.c. of O's serum (kept in ice-chest 5 days) + 0.5 c.c. of a 5 per cent suspension of rabbit's washed corpuscles + rabbit's serum. Some laking in  $1\frac{1}{2}$  hours at  $40^{\circ}$ . Fair laking in 3 hours at  $40^{\circ}$ .

# THE ACTION OF INTRAVENOUS INJECTIONS OF GLANDULAR EXTRACTS AND OTHER SUBSTANCES UPON THE BLOOD-PRESSURE.

BY WALTER W. HAMBURGER.

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OLIVER and Schäfer,<sup>1</sup> in 1894, showed that intravenous injection of a very small quantity of an aqueous extract of the medullary substance of the suprarenal capsule produced a great rise of arterial pressure. Since that time many investigators have tried the effects of various tissue-extracts on the blood-pressure.

In the light of the recent results of Pawlow<sup>2</sup> and others on the activation of trypsinogen by enterokinase, and of Cohnheim<sup>3</sup> on the marked increase which takes place in the glycolytic action of muscle and pancreas when combined, it seemed to me of some interest to try whether one internal secretion might not exert a similar activating effect on another.

With this view I made a considerable number of experiments to determine the mutual action on the blood-pressure of the active substances of various organs and tissues when injected together or successively. I thought it well to begin with the active substance which is best known of all those hitherto investigated in tissue-extracts, viz., adrenalin, and to combine with it a substance capable of exerting by itself a definite and comparatively well-studied action on the blood-pressure, and opposite to the action produced by adrenalin, viz., albumose or peptone.

In addition to these experiments, I injected saline extracts of the kidney, pancreas, liver, thyroid, and pituitary body, singly and combined in various proportions, with varying intervals between

<sup>1</sup> OLIVER and SCHÄFER: *Journal of physiology*, 1894, xvi, p. i.

<sup>2</sup> PAWLOW: *Le travail des glandes digestives*, Paris, 1901.

<sup>3</sup> COHNHEIM: *Zeitschrift für physiologische Chemie*, 1903, xxxix, p. 336.

the time of mixing them and the time of injection. The results, with the exception of the work on the pituitary, are not enough to warrant publication at this time, and will be reserved for a later paper.

In this paper I shall give the results of, (1) experiments upon the simultaneous injection of adrenalin and peptone; (2) experiments upon injection of extracts of the pituitary body; and (3) experiments to determine if possible the mode of action of some of the above-named agents on the lumina of vessels.

PART I. THE EFFECTS OF SIMULTANEOUS AND SUCCESSIVE  
INTRAVASCULAR INJECTIONS OF ADRENALIN AND PEPTONE.

Following the publication of Oliver and Schäfer's paper referred to above, many papers dealing with the same subject appeared, the most important of which were those of Cybulski and Syzmonowicz,<sup>1</sup> Oliver and Schäfer,<sup>2</sup> Syzmonowicz,<sup>3</sup> E. v. Cyon,<sup>4</sup> Lewandowsky,<sup>5</sup> Boruttau,<sup>6</sup> Salvioli and Pezzolini,<sup>7</sup> Salvioli,<sup>8</sup> S. J. and Clara Meltzer.<sup>9</sup> As a result of the endeavors of these experimenters, a number of facts have been definitely established and verified. Of these, the following have a direct bearing on the experiments about to be described.

1. Extracts of the suprarenal substance cause upon intravenous injection a marked contraction of the smaller arteries.

2. A rise of blood-pressure is experienced upon injection of such an extract, even when the vagi are intact. With cut vagi, this rise becomes very much greater.

3. With cut vagi the injection causes augmentation and acceleration.

4. The above results may be obtained by use of any of the following:

- a. Aqueous, saline, alcoholic, or glycerine extracts of the fresh or dried glands
- b. Epinephrin (Abel).
- c. Adrenalin (Takamine).

<sup>1</sup> CYBULSKI and SYZMONOWICZ: Wiener medicinische Wochenschrift, 1896, xlvii, pp. 214, 255.

<sup>2</sup> OLIVER and SCHÄFER: Journal of physiology, 1895, xviii, pp. 230-279.

<sup>3</sup> SYZMONOWICZ: Archiv für die gesammte Physiologie, 1896, lxiv, p. 97.

<sup>4</sup> CYON: *Ibid.*, 1899, lxxiv, p. 97.

<sup>5</sup> LEWANDOWSKY: Centralblatt für Physiologie, 1899, xii, p. 599.

<sup>6</sup> BORUTTAU: Archiv für die gesammte Physiologie, 1899, lxxviii, p. 97.

<sup>7</sup> SALVIOLI and PEZZOLINI: Archives italiennes de biologie, 1902, xxxvii, p. 380.

<sup>8</sup> SALVIOLI: *Ibid.*, 1902, xxxvii, pp. 383, 390.

<sup>9</sup> MELTZER: This journal, 1903, ix, p. 147.

Peptones and albumoses exert on the organism: first, a narcotic action, resembling that of chloroform; second, an anti-coagulant effect, when injected intravenously; and third, an effect on the blood pressure (Schmidt-Mülheim<sup>1</sup> and Pollitzer<sup>2</sup>). The first two actions have no immediate connection with the third (the one with which this paper deals), and therefore will not be considered. W. H. Thompson<sup>3</sup> made a minute investigation of the relation of peptones to blood-pressure, bringing out many interesting points which have been subsequently verified by other physiologists. The following two are of particular interest to the problem in hand:

1. In doses as low as 15-10 mgm., Witte's peptone produces a fall of blood-pressure.

2. All the ingredients of Witte's peptone, with the exception of anti-peptone, possess undoubted vasodilating properties.

With these points as to the contrasted activities of suprarenal extracts and peptones clearly in mind, I shall describe my experiments.

#### METHODS.

In all of my injections I used Parke, Davis, & Company's solution of adrenalin chloride 1-1000, diluted fifty times with 0.9 per cent salt solution. The peptone was injected as a 10 per cent solution of Witte's peptone in 0.9 per cent sodium chloride. As a routine procedure, I used 10 c.c. of each of the above solutions, except where otherwise mentioned. The solutions were injected alone or combined, the proportion of the two constituents being varied at will; they were always made up fresh for each experiment, and when simultaneously used were combined just before the injection took place. The injections were all made on dogs averaging from 9 to 11 kilos in weight. The dogs were anæsthetized with ether, having previously received a hypodermic of a 2 per cent morphine solution in amounts of 1 c.c. for every kilogram of body-weight. The solutions were run into the right femoral vein by hydrostatic pressure. The tracings were taken from the left carotid with the usual mercurial manometer, 2 per cent solution of sodium citrate

<sup>1</sup> SCHMIDT-MÜLHEIM: *Archiv für Physiologie*, 1879, p. 39.

<sup>2</sup> POLLITZER: *Journal of physiology*, 1886, vii, p. 283.

<sup>3</sup> THOMPSON: *Ibid.*, 1896, xx, p. 455; 1899, xxiv, p. 374.

being used as a wash solution for tubes and cannulæ.<sup>1</sup> Before any tracings were taken, the vagi on both sides were cut. In all, twenty-two injections were made.

# RESULTS.

The combined action of adrenalin and peptone is as follows: The first effect noted after the injection of a mixture of the two solutions is an immediate rise of blood-pressure. This is accompanied by the usual augmentation peculiar to adrenalin when the vagi are cut.

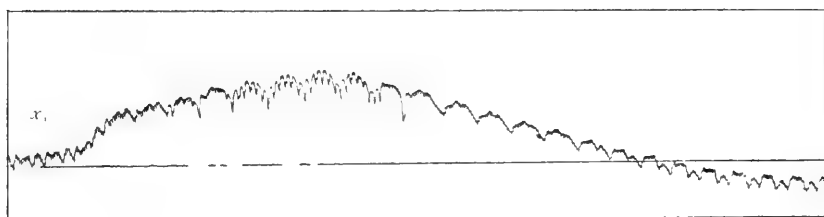


FIGURE 1.—Experiment 16. Table I. November 27, 1903. Left carotid tracing. *x*, point of injection of 10 c.c. of a solution containing 5 c.c. peptone and 5 c.c. adrenalin. Greatest rise, 60 mm. mercury, greatest fall, 22 mm., from the original mean pressure. The straight line is the line of mean pressure at the beginning of the experiment.

This rise is only transient and is immediately followed by a fall of pressure below that at the beginning of the experiment. This fall is accompanied by a weakened heart-beat. Gradually the pressure re-approaches the normal, the low pressure persisting longer, how-

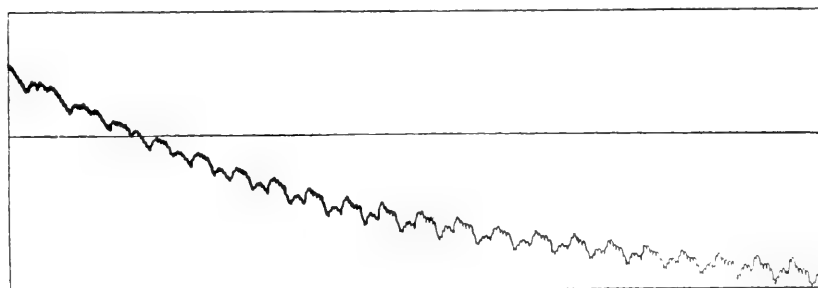


FIGURE 2.—Experiment 17. Table I. November 27, 1903. Left carotid tracing. Shows tracing due to the injection of 15 c.c. of a solution containing 11.25 c.c. peptone and 3.75 c.c. adrenalin. Greatest rise, 48 mm. mercury, greatest fall, 80 mm., original mean from pressure. The figure shows only the last two-thirds of the tracing, the point of injection and initial rise having been cut off.

<sup>1</sup> Eight minutes as an average elapsed between successive injections. This interval sometimes was as short as five minutes, at others prolonged to nine.

ever, than the earlier rise. The amount of fall or rise stands in direct relation to the amounts of adrenalin and peptone injected. Both effects are always present, and may be noted notwithstanding that in some cases a large excess of one or the other constituent is used. Further, the adrenalin effect is always noted first, the peptone fall coming in subsequent to it.

Figures 1 and 2 illustrate the above points. Table I contains the results of five such experiments.

TABLE I.

Experiment number.	ADRENALIN.		PEPTONE.	
	Amount injected in c.c.	Rise in mm. of mercury.	Amount injected in c.c.	Fall in mm. of mercury.
16	5.00	60	5.00	22
17	3.75	48	11.25	80
18	6.375	60	10.625	54
19	5.00	50	10.00	Not taken
20	3.20	44	16.80	50

This table shows a fairly uniform proportion between the amount of substance injected and the fall or rise obtained. The lessening in the absolute amount of fall in the last three experiments is due to general loss of tone of the vascular system, as shown by the persistent low blood-pressure. This was substantiated by the next two experiments, 21 and 22. In these tracings the pressure was so low that the injection of as much as 8.85 c.c. peptone in two successive injections caused no further fall of pressure. On the other hand, concomitant injection of as little as 1.15 c.c. adrenalin caused a demonstrable rise.

The experiments cited above, together with a number of others, show that the blood-pressure responds to a long-continued series of injections of adrenalin better than it does to a similar series of injections of peptone. And further, the action of peptone persists for a much longer time, finally (after several injections of medium or large doses) causing a permanent vasodilatation to such a degree that further injection will not affect the pressure. However, even



when such an extreme vasodilatation is reached, adrenalin always causes a constriction sufficient to raise the pressure to a marked degree. On the other hand, when even a small adrenalin rise was taking place, a large peptone injection caused no lessening of the rise, and the characteristic fall associated with peptone action was not seen until the adrenalin rise had persisted for its normal period.

These differences between the action of adrenalin and peptone are of interest in connection with the question discussed in Part III, whether adrenalin and peptone act on the nervous or on the muscular elements of the vessel wall, and will be referred to again in that part of the paper.

## PART II. THE EFFECTS OF INTRAVASCULAR INJECTION OF EXTRACTS OF THE PITUITARY BODY.

Oliver and Schäfer<sup>1</sup> obtained a rise of blood-pressure on injection of pituitary extracts, occurring more slowly and being maintained longer than that brought about by suprarenal extract. They found, further, that pituitary extract was less active than suprarenal.

Syzmonowicz,<sup>2</sup> who, however, performed only two experiments, obtained results opposed to those of Oliver and Schäfer, viz., a slight lowering of the blood-pressure instead of a rise.

The discrepancy seemed to be explained by Howell's<sup>3</sup> observation that while extracts of the large anterior or hypophyseal lobe caused little or no perceptible change in blood-pressure or heart-rate, extracts of the small posterior or infundibular lobe caused an increase of pressure, accompanied by a slowing of the heart. He showed further that the result of one dose is to diminish or even annul the action of a second.

Schäfer and Vincent<sup>4</sup> came to the following conclusions:

1. The infundibular lobe contains two active substances, a pressor and a depressor, the former soluble in salt solution and insoluble in alcohol and ether, the latter soluble in all these solvents.
2. The characteristic effects of extracts of the infundibular lobe are probably not due to the gray nervous matter of which it is largely composed.
3. The pressor substance acts both upon the heart and upon the

<sup>1</sup> OLIVER and SCHÄFER: *Journal of physiology*, 1895, xviii, p. 276.

<sup>2</sup> SYZMONOWICZ: *Archiv für die gesammte Physiologie*, 1896, lxiv, p. 97.

<sup>3</sup> HOWELL: *Journal of experimental medicine*, 1898, iii, p. 245.

<sup>4</sup> SCHÄFER and VINCENT: *Journal of physiology*, 1899, xxv, p. 87.

peripheral arteries. Its action is a prolonged one; the action of the depressor is evanescent.

4. The pressor effect may be accompanied by cardiac slowing.

In addition, Osborne and Vincent,<sup>1</sup> Halliburton,<sup>2</sup> Schäfer and Magnus,<sup>3</sup> and Golla<sup>4</sup> have experimented upon injections of the gland, but their results have no direct bearing upon the subject as I have taken it up.

#### METHODS.

The methods followed were similar to those employed in the work described in Part I, except as to the nature of the solvents. The fresh material was obtained as needed from the skulls of oxen, through the courtesy of Swift and Company, Union Stock Yards. Saline extracts at room-temperature were separately made of the infundibular and hypophyseal lobes, and were injected singly and combined with each other. The two lobes were carefully isolated, particular care being taken to separate cleanly the infundibular process (the stalk uniting the infundibular lobe to the infundibulum proper) from the enveloping hypophysis. This point of most intimate union between the two lobes was, for a number of experiments (see Table V), excised, with a safe margin of tissue on either side, and the remnants of the two lobes, now free beyond question from mutual contamination, as regards the morphological elements at any rate, were extracted singly.

Besides the saline extracts, glycerine, alcoholic, and ether extracts of the dried material were made at room-temperature. The alcohol and ether extracts were made as follows: dried hypophyseal and infundibular lobes were extracted with 95 per cent alcohol, the extract filtered, and the alcohol allowed to evaporate. The residue was then treated with ether, which took up a portion of it and left a portion in the dish. This second residue, after decanting off the supernatant ether solution (see below), dissolved readily in 0.9 per cent salt solution, the saline extract of hypophysis constituting solution No. 1 (to be referred to later, Table IV), while the saline extract of infundibulum constituted Solution No. 2.

The ether solution was then filtered, and the filtrate evaporated, the

<sup>1</sup> OSBORNE and VINCENT: *British medical journal*, March 3, 1900, p. 38.

<sup>2</sup> HALLIBURTON: *Journal of physiology*, 1901, xxvi, p. 229.

<sup>3</sup> SCHÄFER and MAGNUS: *Journal of physiology*, 1901, xxvii, p. ix.

<sup>4</sup> GOLLA: *Lancet*, Feb. 15, 1902, p. 442.

residue being suspended in 0.9 per cent salt solution, in which it dissolved but slightly. This saline suspension of hypophyseal material constituted Solution No. 3; that of the infundibular lobe, Solution No. 4.

In all, thirty-six injections were made.

#### RESULTS.

The results are given in Tables II-V.

In Experiment 1, Table II, is seen a characteristic fall produced by injection of 10 c.c. of a saline extract of the hypophyseal lobe. The blood-pressure fell from 126 to 58 mm. of mercury in thirty-four seconds after the start of the injection — thirteen seconds being con-

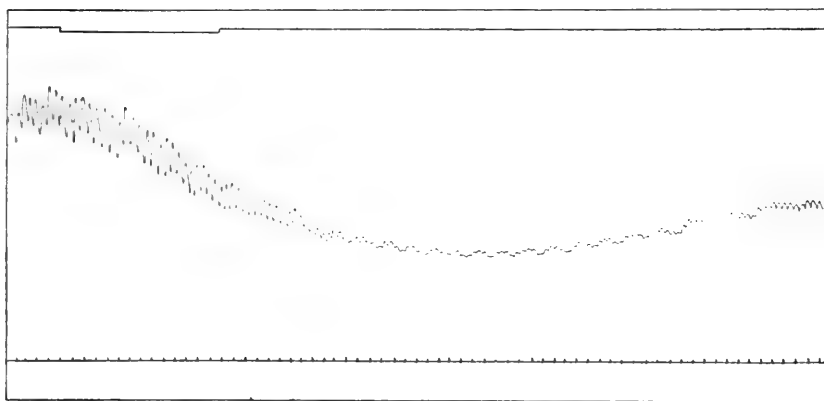


FIGURE 3.— Experiment 1. Table II. February 2, 1904. Left carotid tracing. 10 c.c. saline extract hypophysis injected. Pressure fell from 126 mm. Hg to 58 mm., and then rose to 88 mm. Pulse at start, 108. After injection, 132. Note weakened heart action. Time marks on base line mark seconds.

sumed in the injection of the solution. This fall was accompanied by an acceleration of from 132 to 108 beats per minute, together with a weakened heart action. Figure 3 illustrates this experiment. Similar results are shown in Experiment 1, Table III, the fall, however, being succeeded by a slight rise of 4 mm., which may be due to a specific pressor substance or simply to the reaction of the vessels following the great fall of 46 mm.

Experiment 2, consisting of an injection of infundibular extract, followed Experiment 1, producing a marked rise in blood-pressure. The pressor effect and slowing of the pulse agree with Schäfer and Vincent's results.<sup>1</sup>

<sup>1</sup> SCHÄFER and VINCENT: *Loc. cit.*

TABLE II.

Experiment No.	Extract injected.	Mean pressure in mm. of Hg before injection.	Initial rise in mm. of Hg to	Initial fall in mm. of Hg to	Secondary rise in mm. of Hg to	Secondary fall in mm. of Hg to	Additional notes.
1 <sup>1</sup>	10 c.c. Hypophysis	126	..	58	88	..	Heart weakened and accelerated from 108 to 132 beats per minute. As effect wore off, beat became slower and stronger.
2	10 c.c. Infundibulum	63	82	68	119	..	Heart very feeble at start of experiment, was strengthened and slowed after injection. Pressure continued to rise after tracing was stopped.
3	10 c.c. Hypophysis	75	No change	No change	..	..	Respiration very irregular during this injection. Heart slowed and strengthened.
4	10 c.c. Infundibulum	71	87	..	..	..	Respiration irregular. Beat slightly strengthened.
5	10 c.c. { Hypophysis	81	99 <sup>2</sup>	64	76	..	Secondary rise came very slowly, 1 1/2 minutes after injection.
6	25 c.c. { Infundibulum	75	110 <sup>2</sup>	..	..	..	Rise due to mechanical increase in volume of circulating fluid. See below.
7	25 c.c. 0.9 percent NaCl [control injection]	73	93 <sup>2</sup>	..	..	..	Rise due to increase in volume of circulating fluid.

<sup>1</sup> Immediately following the first injection, the heart very nearly stopped beating, and the blood-pressure became very low, respiration being absent. Artificial respiration was kept up for 20 minutes, until natural respiration began again. Administration of adrenalin during this period had an unusually short transitory effect on the pressure.

<sup>2</sup> Rise was caused by mechanical increase of volume of circulating fluid by injection of large volume of liquid. It is convenient to speak of such a rise as an "injection rise" (see Fig. 4).

TABLE III.

Experiment No.	Extract injected.	Mean pressure in mm. of Hg before injection.	Initial rise in mm. of Hg to	Initial fall in mm. of Hg to	Secondary rise in mm. of Hg to	Secondary fall in mm. of Hg to	Additional notes.
1	10 c.c. Hypophysis	88	..	42	92	..	Secondary rise was very gradual. Heart was weakened and accelerated. Pulse, before experiment, 102; after experiment, 114. Heart weakened and accelerated.
2	10 c.c. Infundibulum	87	98	..	..	63	Secondary fall was very gradual. Fall succeeded by steady, gradual rise to normal. Heart slightly accelerated though still very slow.
3	10 c.c. Infundibulum	62	..	38	62	..	Control experiment to contrast adrenalin and infundibular effects.
4	10 c.c. Adrenalin (1-50,000)	103	191	..	..	87	Heart-beat unchanged. Secondary rise steady and gradual. Beat strongly accelerated.
5	10 c.c. Hypophysis	75	No change	No change	..	..	Secondary rise slow and steady. Slight acceleration.
6	10 c.c. Infundibulum	84	85	68	88	..	Latent rise slight. Due to salt solution.
7	5 c.c. } Hypophysis	90	93	78	106	..	No marked changes.
8	10 c.c. } Infundibulum	98	No change	No change	104	..	
9	5 c.c. Hypophysis	101	No change	No change	..	..	

Experiment 2, Table III, shows, after the rise of pressure produced by injection of infundibular extract, an interesting secondary fall, which may be caused by the presence of a depressor substance. Such a secondary fall is analogous to the combined effect of the simultaneous injection of chemically distinct pressor and depressor substances (adrenalin and peptone), investigated in Part I of this paper.

In Experiment 3, Table II, a second injection of hypophyseal extract produced no effect on the blood-pressure, as always happens

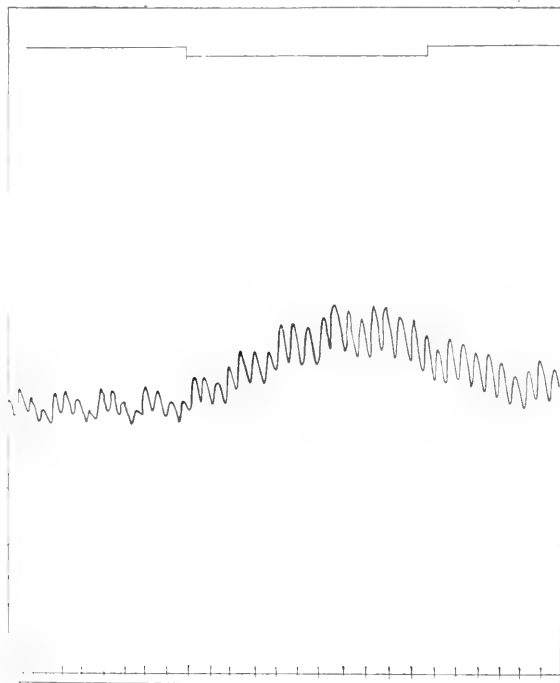


FIGURE 4.—Experiment 7. Table II. Left carotid tracing. 25 c.c. 0.9 per cent solution of sodium chloride injected as a control to show the mechanical "injection rise" (Experiment 7, Table II).

if the second injection is made immediately after the first. The same result was obtained in Experiment 6, Table II, and in Experiments 5, 8, and 9, Table III.

This absence of change in the pressure is analogous to a similar phenomenon noticed in the case of the infundibular lobe by Schäfer and Vincent, on injection of repeated doses of infundibular extracts. Sometimes, however, they obtained a *fall* of pressure instead of the

TABLE IV.

Experiment No.	Material injected. <sup>1</sup>	Mean pressure in mm. of Hg before injection.	Initial rise in mm. of Hg to	Initial fall in mm. of Hg to	Secondary rise in mm. of Hg to	Secondary fall in mm. of Hg to	Additional notes.
1	10 c.c. saline extract	94	No change	No change	..	..	
2	colloid material <sup>2</sup> . . . 10 c.c. Solution No. 1 (Hypophysis) . . .	99	..	81	118	..	Pulse, at beginning, 120 per minute; after injection, 102; beat weakened.
3	10 c.c. Solution No. 1 (Hypophysis) . . .	124	..	96	137	..	Pulse, at beginning, 86; after injection (initial fall), 96; secondary rise returned to 86.
4	10 c.c. Solution No. 2 (Infundibulum) . . .	114	..	92	121	..	Pulse, before experiment, 72; during initial fall, 78; during secondary rise, 72.
5	10 c.c. Solution No. 2 (Infundibulum) . . .	107	..	72	113	..	Heart-beat weakened.
6	10 c.c. } 5 c.c. No. 1 . . . 10 c.c. } 5 c.c. No. 2 . . .	109	..	95	..	..	
7	10 c.c. Solution No. 3 (Hypophysis) . . .	96	No change	No change	98	..	
8	10 c.c. Solution No. 3 (Hypophysis) . . .	93	..	83	99	..	
9	10 c.c. Solution No. 3 (Hypophysis) . . .	88	..	77	97	..	
10	10 c.c. Solution No. 4 (Infundibulum) . . .	87	..	50	97	..	Heart-beat slower.
11	10 c.c. Solution No. 4 (Infundibulum) . . .	90	..	47	104	..	
12	10 c.c. } 5 c.c. No. 3 . . . 10 c.c. } 5 c.c. No. 4 . . .	102	..	63	108	..	
13	10 c.c. } 3 c.c. glyc. ext., hypophysis, 7 c.c. 0.9% NaCl	98	..	85	91	69	See page 295 for explanation of Experiments 13 and 14.
14	10 c.c. } 5 c.c. glyc. ext., hypophysis, 5 c.c. 0.9% NaCl	77	..	49	66	52	

<sup>1</sup> See pages 288-289 for description of methods of preparations of Solutions Nos. 1, 2, 3, and 4, used in this table.<sup>2</sup> For description of this colloid material, see page 294.

pressor effect which the first dose gave. They explain the depressor action by the presence of a specific depressor substance in the posterior lobe. I shall refer again to this phenomenon in the case of the anterior lobe, in mentioning some later experiments. In Experiment 4, Table II, a second injection of infundibular extract gave but a slight rise of 16 mm. Hg, while a rise of 56 mm. was caused by the first injection. In Experiments 3 and 6, Table III, a second injection was followed by an initial fall, with no pressor effects whatever.

The experiments in Table IV were undertaken to determine if possible whether the active principle which the previous work had shown to exist in the anterior lobe could be approximately isolated with alcohol and ether. Such was found to be the case, and in addition a number of interesting accessory features were revealed.

A saline extract of the peculiar orange-yellow, semi-translucent, hyaline substance often found in varying amounts between the two lobes was injected, with negative results (Experiment 1, Table IV). This colloidal substance was sometimes in a liquid, extremely viscous, and gelatinous state, while in other instances it had solidified to form a firm plate which followed the contour of the contiguous lobes. It was found to be practically insoluble in cold or boiling acid and alkali, as well as alcohol, ether, chloroform, etc.

In Experiment 2, Table IV, Solution No. 1 of the hypophyseal lobe caused a measurable fall (18 mm.), and a subsequent rise to 118 mm. of pressure. This rise is of interest, inasmuch as an analogous rise was obtained in Experiment 1, Table III, by using the saline extract. I immediately injected a second dose of Solution No. 1 (Experiment 3, Table IV), and instead of getting no change in pressure, as in the case of the saline extract, the pressure fell 28 mm., and was followed by a rise to 137 mm. Apparently, then, the second injection of an alcoholic extract of the dried lobe, after the removal of those portions of it which are soluble in ether, will produce a fall of pressure, even when made immediately after the first. These experiments, like Experiment 1, Table III, already discussed, may perhaps indicate the presence of a pressor substance whose action in this case manifests itself subsequently to the fall, instead of, as is usual, preceding it. The ability of this alcoholic extract to produce a fall of pressure on successive injections may indicate that some "inhibitory substance" present in the saline extract had been eliminated. Moreover, the coincidence that this fall produced by injection of the alcoholic extract was succeeded by a rise, may point to an intimate relation



between the absence of this inhibitory substance and the pressor effect.

Experiments 4 and 5, Table IV, confirm Schäfer and Vincent's statement that the pressor substance is insoluble in alcohol and that the depressor effect may be obtained on repeating the injections.

Experiments 7-9, Table IV, show no *characteristic* fall on injection of Solution No. 3, though there is a *slight* fall in the case of the last two. The depressor substance is, therefore, apparently absent from this solution, being insoluble in ether and soluble only in alcohol and salt solution. Of course the possibility must be granted that a depressor substance might be present in the solution, and yet be inactive in an animal submitted, as this dog was, to repeated previous injections of Solutions 1 and 2, just as we find that the saline extract was inactive on a second injection.

The ether-soluble part of the alcoholic extract of the infundibulum (Solution 4) produces the characteristic depressor fall, as shown by Experiments 10 and 11, Table IV, this fall being more marked, if anything, than the fall produced by the alcoholic, ether-insoluble portion (confirmatory of Schäfer and Vincent, though a new detail in technique is added, viz., the separation of the ether-soluble and ether-insoluble substances of the alcoholic extract).

In Experiments 13 and 14, Table IV, glycerine extracts were used simply for the sake of completeness. The initial fall, which occurs very abruptly, is due to the glycerine alone, as shown by control experiments (Halliburton<sup>1</sup>), while the subsequent secondary fall is due to the depressor substance extracted by the glycerine.

The experiments in Table V were undertaken to decide whether the depressor effect invariably produced by the anterior lobe was due to intrinsic substances in its cells and not to any possible mixture with the depressor substances of the posterior lobe.

Experiment 1, Table V, shows that the injection of a saline extract of the anterior lobe from which the posterior portion in contact with the infundibular lobe had been removed, produces a characteristic fall of 28 mm. of mercury, thus forcing the conclusion that the active agent is an integral constituent of the lobe in question. Two successive injections of the same solution, given immediately, failed to produce a fall, entirely in accord with many other similar trials described earlier.

<sup>1</sup> HALLIBURTON: *Loc. cit.*

TABLE V.

Experiment No.	Extract injected. <sup>1</sup>	Mean pressure in mm. of Hg before injection.	Initial rise in mm. of Hg to	Initial fall in mm. of Hg to	Secondary rise in mm. of Hg to	Secondary fall in mm. of Hg to	Additional notes.
1	10 c.c. Hypophysis	80	..	52	..	..	Pulse unchanged, heart slightly weakened.
2	10 c.c. Hypophysis	78	No change	No change	..	..	
3	10 c.c. Infundibulum	84	109	53	88	..	Pulse and respiration markedly affected, very weak during fall; slow and labored during secondary rise.
4	10 c.c. excised portion <sup>2</sup> (parts of both lobes)	72	No change	No change	..	..	
5	10 c.c. excised portion <sup>2</sup> (parts of both lobes)	79	No change	No change	..	..	
6 <sup>3</sup>	10 c.c. Hypophysis	96	No change	No change	..	..	
7 <sup>3</sup>	10 c.c. Hypophysis	78	..	68	..	..	

<sup>1</sup> The material used in these experiments was prepared free from morphological contamination of one lobe or the other by the excision of the point of most intimate union between the two lobes (see page 288).

<sup>2</sup> The injection of the excised portion has no particular significance. It was instituted more as a control than anything else.

<sup>3</sup> Between Experiments 6 and 7, twenty-five minutes were allowed to elapse, no experimental work being done on the animal during that time. The pulse was slightly increased and the blood-pressure slightly decreased during this period (see page 297).

Following the last of these injections, twenty-five minutes were allowed to elapse, in order to see whether a fall could then be produced by a further injection. During this period the pulse was slightly increased and the pressure slightly decreased. A fourth injection of hypophyseal extract was now made, with the result that a distinct fall of 10 mm. of pressure was recorded, lasting for three-fourths of a minute.

This suggests again that some definite substance is present along with the depressor substance, which prevents a second injection (given immediately) from producing an effect, and further, that this "inhibitory" substance is slowly removed (by oxidation or other process) from the blood-stream, or at least loses its "inhibiting" influence if allowed to remain for a certain period in the body. And, as shown earlier, this inhibitory substance is not present in the ether-insoluble part of the alcoholic extract of the dried lobe (Solution No. 1). The fact that this inhibitory substance always manifests itself in successive injections of the hypophyseal lobe except in (1) successive injections of Solution No. 1, and in (2) injections of saline extracts when a considerable time interval has elapsed between successive injections, may explain why previous investigators failed to get a depressor effect. In view of the fact, however, that no details are given in the papers quoted, regarding the experiments on the hypophyseal lobe, the above explanation can merely be offered as a suggestion.

I now wish to speak briefly of the results of the injection of combined extracts of the two lobes. In Experiment 5, Table II, and Experiment 7, Table III, injections of a mixture of saline extracts of both lobes were made. No decided results were obtained, there being a slight initial rise followed by a measurable fall in each case, the sequence of which conforms to that of adrenalin and peptone.

In Experiment 6, Table IV, a combined solution of Extracts No. 1 and No. 2 was used, and in Experiment 12, Table IV, a combined solution of Extracts No. 3 and No. 4. In each case a decided fall was observed, which might be explained by the absence of the "inhibitory substance," as well as the pressor substance.

### PART III. DO ADRENALIN AND PEPTONE ACT ON THE NERVOUS OR MUSCULAR APPARATUS OF THE VESSEL WALL?

When any substance capable of affecting the blood-pressure is injected intravenously, it may cause a change in the lumen of the

vessel by (1) setting up a reflex, thus involving the vasomotor centres in the medulla; (2) direct stimulation of vasomotor nerve endings in the vessel wall; or (3) direct stimulation of the muscular elements of the vessel wall without the intervention of any nerve ending or fibre.

Schäfer and Oliver<sup>1</sup> in regard to suprarenal extract, write as follows: "That the contraction of the arterioles is due to direct action of the active principle of the gland (suprarenal) upon the muscular tissue of the blood-vessels is shown by the experiment of perfusing through the blood-vessels of the frog Ringer's circulating fluid containing suprarenal extract *after* the nervous system had been destroyed; it is also demonstrated by the fact that it occurs equally well after section of the spinal cord, and after section of the nerves going to the limb."

Against this view Cybulski and Syzmonowicz,<sup>2</sup> and also E. v. Cyon,<sup>3</sup> state that the marked rise of blood-pressure is due to stimulation of the vasomotor centres, and not to direct action on the vessels.

Lewandowsky,<sup>4</sup> however, showed that the intravascular injection of suprarenal extract produced a contraction of smooth muscles of the eye (cat), even after the connection with the central nervous system had been destroyed by excising the superior cervical and jugular ganglia, attended with concomitant degeneration of nerve fibres and endings. Oliver and Schäfer's hypothesis was further substantiated by Boruttau,<sup>5</sup> although working along other lines, and again by Salvioli<sup>6</sup> in 1902.

S. J. and Clara Meltzer,<sup>7</sup> in 1903, removed the superior cervical ganglion in rabbits, and cut the third cervical nerve and its connecting branches, thereby depriving the blood-vessels of the ear on the operated side of all central innervation. On the day of the operation, and again, after an interval of seven days, they injected adrenalin intravenously, with the following results:

1. The degree of constriction which blood-vessels attain under the influence of adrenalin in the ear on the operated side is about the same as that of the ear on the normal side.

<sup>1</sup> OLIVER and SCHÄFER: *Journal of physiology*, 1895, xviii, p. 230.

<sup>2</sup> CYBULSKI and SYZMONOWICZ: *Loc. cit.*

<sup>3</sup> CYON, E. v.: *Loc. cit.*

<sup>4</sup> LEWANDOWSKY: *Loc. cit.*

<sup>5</sup> BORUTTAU: *Loc. cit.*

<sup>6</sup> SALVIOLI: *Archives italiennes de biologie*, 1902, xxxvii, p. 350.

<sup>7</sup> MELTZER: *Loc. cit.*

2. The constriction, however, sets in later, and develops more slowly on the operated side than on the non-operated side.

3. On the normal side constriction is usually followed by a moderate but distinct vasodilatation. Such an effect is absent on the operated side.

4. On the operated side constriction lasts longer than on the non-operated side.

In a later communication<sup>1</sup> they say that in normal rabbits a large distinctly poisonous dose of adrenalin, injected subcutaneously, causes blanching of the ears; a medium dose causes a moderate but distinct dilatation of the blood-vessels. In rabbits in which the vasomotor nerves were cut on one side, a medium dose induced a distinct contraction of the vessels on the operated side, and a dilatation on the non-operated.

In a preliminary report<sup>2</sup> of experiments elaborating the work of Lewandowsky on the dilatation of the pupil produced by suprarenal extracts, the same observers stated the results of subcutaneous injection and subconjunctival instillation of adrenalin upon the pupils of rabbits whose superior cervical ganglion had been removed. They found that subcutaneous injection or installation into the conjunctival sac of a medium dose produces a maximum dilatation on the ganglion-free side, but no dilatation on the normal side. They showed further that the entire ganglion must be removed, for if only two-thirds are removed, or if only the connecting nerves be severed, adrenalin produces no such effect.

Brodie and Dixon<sup>3</sup> have likewise recently published some observations on the action of suprarenal extract which have a bearing on the subject. In their work on the innervation of the pulmonary blood-vessels, they reach the following conclusions:

1. While barium chloride produces a constriction of the pulmonary vessels similar to that observed in the systemic vessels, adrenalin, pilocarpine, and muscarine produce a dilatation.

2. Barium chloride acts directly on muscle fibre. Adrenalin, pilocarpine, and muscarine excite constriction by acting on nerve-endings.

The animals used by me were rabbits. The superior cervical

<sup>1</sup> MELTZER: This journal, 1903, ix, p. 252.

<sup>2</sup> MELTZER: Centralblatt für Physiologie, 1904, xvii, p. 651.

<sup>3</sup> BRODIE and DIXON: Journal of physiology, 1904, xxx, p. 476.

ganglion was completely removed on one side with from one to one and a half centimetres of attached sympathetic nerve. The ganglion and nerve on the other side were either left undisturbed or the nerve simply cut across. In the latter case, a normal, unoperated rabbit served as a control.

In an animal whose superior cervical ganglion had been completely removed nine days before, on the right side, and whose left side had not been disturbed, I dropped adrenalin  $\frac{1}{50000}$  into the right eye. The conjunctival surface of both upper and lower eyelids, as well as the sclerotic vessels, became pale instantly—having been normal before. The eye was immediately washed with 0.9 per cent sodium chloride solution and a 10 per cent solution of peptone added. The eye remained pale. Paleness persisted for from seven to ten minutes, at the end of which time the normal color could be seen. This was repeated at intervals of from fifteen to thirty minutes, with constant results in each case. The same results were obtained in similar experiments fourteen days after the operation.

As a control, the above experiments were exactly repeated on the left eye and on the eyes of an unoperated animal, with similar results, except that an application of peptone subsequently to the application of adrenalin was accompanied by a distinct flushing of the vessels of the eye and eyelid.

The above experiment was also tried five days after the operation on a rabbit whose superior cervical ganglion had been removed on the left side, and whose sympathetic nerve had been severed on the right side. Adrenalin produced a paling in the right eye, and after removal of the adrenalin with salt solution, peptone caused an immediate flushing. On the left side (ganglion-free), the same paling was observed as before, but repeated peptone administration had no effect on the local anæmia, no flushing being observed until ten or fifteen minutes had elapsed. The above experiments were repeated several times on this same day, and also ten days after, and again fourteen days after operation, with confirmatory results on each occasion.

Fourteen days after the operation, the flushing of the right eye upon the introduction of peptone was less marked, and required a longer time to take place than five days after the operation, and the flushing was slower than that obtained from a control unoperated animal.

Extracts of hypophyseal lobe of the pituitary body of the ox caused marked flushing in both eyes, while extracts of the infundibular lobe caused a slight flushing on the right side and none on the left.

From these results, we may conclude that the nerve elements of the vessel wall are not necessary for its constriction by adrenalin, since it produced its effect in regions where the nervous control had been eliminated by the extirpation of the ganglion from five days to two weeks previously. On the other hand, such nerve elements seem to be necessary for the effects of peptone to become manifest, inasmuch as the vessels of regions in which the nerve fibres and endings may be assumed to have degenerated, or at least to have suffered important functional and structural change, could not be caused to dilate upon its administration.

The effects of the pituitary extracts were not particularly strongly marked, and were inconstant in comparison with the action of adrenalin and peptone. This apparent indefiniteness may be explained by the simultaneous presence of the pressor and depressor substances in the one solution.

As confirmation of Meltzer's work, I might say that with adrenalin an extreme dilatation of the pupil was always seen first, and was most pronounced on the side from which the ganglion had been completely removed, the other pupil sometimes remaining perfectly normal. At such a moment, the pupil on the ganglion-free side showed no reaction to light, while the pupil of the normal eye showed a constriction followed by a quick dilatation when the light was shut off.

The fact that the peptone solution always produced an effect on the unoperated animal is in accord with the conclusions reached by Chittenden, Mendel and Henderson,<sup>1</sup> and independently by Ledoux<sup>2</sup> and Thompson<sup>3</sup>: that although the anti-coagulable power of peptone may be due to the subsequent formation by the liver or other organ of a second substance, the depressor effect is due to the constituents of the peptone itself.

#### SUMMARY OF RESULTS.

**Part I. — I.** In a simultaneous intravenous injection of adrenalin and peptone, the adrenalin rise is present first, and is succeeded by the peptone fall. This rise and fall always occur in a regular sequence, and may be detected, though the amount of either constituent be enormously in excess of the other.

<sup>1</sup> CHITTENDEN and OTHERS: This journal, 1899, ii, p. 142.

<sup>2</sup> LEDOUX: Archives de biologie, 1896, xiv, p. 63.

<sup>3</sup> THOMPSON: *Loc. cit.*

2. The adrenalin rise appears after a very short latent interval, and is transitory. The peptone fall occurs more slowly, persists longer, and soon becomes permanent. Administration of peptone immediately after an adrenalin injection will in no way interfere with the extent or duration of the rise. On the contrary, the administration of adrenalin subsequent to a peptone injection is adequate to check the fall and produce a normal adrenalin rise.

**Part II.** — 1. The intravenous injection of a saline extract of the hypophyseal (anterior) lobe of the pituitary body produces a distinct fall of blood-pressure. This fall is accompanied usually by an acceleration and weakening of the heart.

2. A second injection of a saline extract of hypophyseal lobe, immediately following the first, fails to produce any change in blood-pressure. If a considerable interval be allowed to elapse, a second injection will produce a fall.

3. The active depressor substance is soluble in alcohol, glycerine, and salt solutions, but insoluble in ether. Repeated doses of the alcoholic extract, following each other immediately, are active.

4. A secondary rise above the normal follows the depressor effect produced by alcoholic extract of the hypophyseal lobe. It is also sometimes seen after injection of a saline extract. This may be due to an active pressor substance present in small amounts in the hypophyseal lobe, or to the elimination of the "inhibitory" substance, present in the saline extract.

**Part III.** — 1. The experiments on the eyes of rabbits support the view that adrenalin produces its constrictor effect by acting directly on the musculature of the vessel wall.

2. Witte's peptone causes dilatation of the vessels of the eye when applied directly, by acting on the vasomotor nerve-endings alone. This effect is produced by an immediate action of some of its constituents, and not by a new substance formed under the influence of the body, as is the case with its effects on the coagulation of the blood.

In conclusion, I desire to express my appreciation and thanks to Professor Stewart, under whose direction this work was pursued, for invaluable advice and criticism.



## METHODS FOR THE QUANTITATIVE CHEMICAL ANALYSIS OF THE BRAIN AND CORD.

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A CONSIDERATION of the various quantitative chemical analyses of brain tissues, so far recorded in the literature, indicates most plainly that methods involving separation by means of solvents can be of value only for the isolation of groups of substances. Only under most exceptional conditions can these methods be carried to such refinement as to bring about the complete separation of two chemical individuals without loss of material. In mixtures containing a large number of compounds, the influence which one substance exerts on the solubility of another is so great that the solubility of a substance when pure is no criterion of its behavior in a mixture. It becomes necessary, therefore, to devise for each constituent a method depending on some radicle or group of atoms characteristic of itself and not found in any one of the other constituents. If, however, two or more constituents have the same radicle, they must be determined as a group, and then distinguished from one another by certain other characteristics.

After all the constituents of a tissue have been determined by methods, devised as outlined above, and the total adds up to or nearly to 100 per cent, so that there is reason to believe that everything has been accounted for, it is necessary further to check the result by making a determination of the total phosphorus, nitrogen, and sulphur in, for instance, the alcohol-ether-soluble portion of the tissue. The amount of phosphorus, nitrogen, and sulphur represented by each constituent of the tissue belonging to the alcohol-ether-soluble portion is then calculated with either the structural formula or the elementary analysis of the substance as a basis. After everything has been accounted for, the *total calculated* phosphorus, nitrogen, and sulphur should check the *total found* phosphorus

nitrogen, and sulphur within the limits of error of the determination. If, on the other hand, all the elements are not accounted for in terms of compounds, or the total of the constituents does not add up to 100 per cent, a consideration of the elements not accounted for will then give some clue as to the nature of the compound or compounds remaining to be isolated. The same principle can then be applied to that portion of the tissue insoluble in alcohol and ether.

A separation of the anatomical elements of a tissue preparatory to chemical analysis would indeed be very desirable, but is evidently an impossibility. Such elements as blood-vessels, connective tissue, and, if possible, the blood should, however, be removed wherever feasible. The brain represents a most favorable tissue for such work, as all the large blood-vessels can easily be removed with the pia mater, and there is very little connective tissue. In an animal it is easily possible to remove all the blood by transfusing salt solution. (In the human subject this is hardly possible, and the blood thus represents the most serious source of error in comparative work.) After removal of the blood, there remain, then, in the cortex, for example, neuroglia cells, some blood-vessels, medullated and non-medullated nerve fibres, and the various types of nerve cells. If in such a tissue chemical differences were to be observed as a result of physiological nerve activity, it would seem reasonable to refer these differences to changes in the nerve cells, rather than to the neuroglia cells or the cells in the blood-vessel walls, which can be assumed to undergo less active metabolism. In the study of pathological material, in which proliferation of neuroglia cells is liable to have taken place, this source of error must be as nearly as possible determined by microscopic examination, which should be made in any case. The substances introduced by the presence of medullated nerve fibres can be approximately determined by an analysis of a pure mass of medullated fibres as found in the corpus callosum. Any attempt to obtain cortical grey matter free from medullated fibres would evidently be useless.

The results to be expected from such a quantitative study of the brain are as follows:

1. It may be possible to obtain some clue as to the physiological significance of the various substances by following out the time at which they appear during embryological development<sup>1</sup> of the tissue,

<sup>1</sup> S. HATAI has undertaken this part of the work in the Neurological Laboratory of the University of Chicago.

and the ease with which they disappear as the result of prolonged chronic pathological changes,<sup>1</sup> correlating the results thus obtained with variations of functional activity.

2. A study of the relation of the various substances to one another, and to their intermediate and ultimate decomposition products, may give some insight into the obscure processes of the metabolism of the nerve cell.

3. Variations from the normal, in the proportion of the various constituents, produced by such acute changes as lack of oxygen or the action of drugs, may be the means of giving a chemical basis for pharmacological investigation.

Results obtained in this way can then be further correlated by a study of the distribution of the various constituents by microscopic methods. At present such methods have not been sufficiently developed from the chemical side to be of much value.

#### METHOD OF COLLECTING THE MATERIAL.

As there is no fluid in which such a tissue as the brain can be preserved as a whole, for quantitative chemical work, without introducing serious sources of error, it becomes necessary to weigh the material as soon as collected, and to determine immediately the amount of water.

**White matter.**—The method of procedure for white matter is as follows: The corpus callosum is used as representing the largest amount of pure white matter to be found in the central nervous system. It is freed as much as possible from blood-vessels, and then cut into slices, which are immediately placed in weighed glass stoppered bottles. At the same time somewhat thinner slices are placed between weighed watchglasses (fitted with a clip) for the water determination. This insures that the material used for extraction is in about the same state of hydration as that used for the water determination. It is best not to incur the risk of the material becoming too dry, in any attempt to prepare it in a fine state of division for extraction. As will be described later, after the second or third alcohol and ether extraction, the material can be pulverized in a mortar much finer than it could possibly be cut in the moist state. For the water determination from 1.0 gm. to 1.5 gm. should be taken. For the extraction it is better not to take less than 5 gm. and a larger

<sup>1</sup> This investigation is being carried on in this laboratory with material collected at the Pathological Laboratory of the Claybury Asylum, London.

amount than 10 gm. would seriously interfere with the completeness of the extraction. If more material than 10 gm. is available, it is better to collect another sample and place it in a second bottle. The water determination should be made immediately, to insure the material against decomposition. The extraction can be postponed by preserving the material in three times its weight of absolute alcohol. As the weight of the moist material is known, and the amount of water determined in the separate sample, this method of preservation introduces no source of error, as the alcohol in which the material is kept is also used in the first extraction.

**Grey matter.** — The largest aggregation of grey matter in the human brain is to be found in the cortex, although spread over a considerable area. As all the layers of the cortex, even the uppermost ones, contain medullated nerve fibres, it is useless to attempt the collecting of pure grey matter. In fact, for the investigation for which this work is to be a basis, it is essential to obtain all of the cortex, and not merely to shave off the top layers in an attempt to avoid the white matter. The best method, as suggested by Dr. Watson, was found to be as follows: Sections about 4 mm. thick are cut as much as possible perpendicular to the convolutions, and at right angles to the fissures. The easily visible layer of grey matter which, if the section is exactly perpendicular to the convolution, is of nearly the same thickness on both sides, is then trimmed off by running a knife as close as possible along the line of division between the grey and the white. Before placing in the weighed bottle, this strip is examined carefully, and any adhering white matter snipped off with a fine pair of scissors. The advantage of this method is that the white matter is always exposed to the view, and that if any small particles do adhere, they can easily be seen and removed. The water determination, the weighing and preserving are done exactly as described for the white matter. In order to localize somewhat for future work the place from which the material was taken, two and occasionally three parts of the cortex were always used.

I. **The prefrontal area.** — Including approximately the anterior two-thirds of the first frontal, and corresponding portion of the median surface of the cortex, the anterior two-thirds of the second frontal, and the anterior third of the third frontal convolutions.

II. **The motor area.** — Ascending frontal as far as the bottom of the fissure of Rolando, and neighboring parts in front, including Broca's convolution.

III. **The visuosensory area.**—Lower lip of the anterior calcerine fissure, both lips and surrounding parts of the posterior calcerine fissure, and a small portion of outer aspect of occipital pole.

For microscopic examination, samples were taken from these regions. One was preserved in Müller's fluid, after hardening in formol for determining the amount of degeneration in the fibres by Marchi's method, and the amount of wasting by the Pal Weigert-method. Another sample, after hardening in formol, was dehydrated with alcohol and imbedded in paraffine for studying the changes in the neuron cell with Nissl's methylen blue method.

#### CHEMICAL CONSTITUENTS OF BRAIN-TISSUE.

The substances so far isolated from the brain which must be considered in devising quantitative methods of determination, as outlined in the introduction, are:

1. **Water**,  $H_2O$ , present in largest amount.
2. **Simple and compound proteids** [C, H, O, N, S, P].  
*Globulin* coagulating at  $47-50^{\circ} C.$  (Halliburton<sup>1</sup>).  
*Globulin* coagulating at  $70^{\circ} C.$  (Halliburton).  
*Neurostromin* (Schkarin<sup>2</sup>): extracted by sodium hydrate, present only in small amount.  
*Nucleoproteid* (Levene<sup>3</sup>): contains 0.57 per cent phosphorus. The nuclealbumin of Halliburton<sup>1</sup> and the neuroglobulin of Schkarin<sup>2</sup> may be considered to be identical or closely related to the compound isolated by Levene.  
*Neurokeratin* (Kühne and Chittenden<sup>4</sup>): an albuminoid insoluble in sodium hydrate and not digested by ferments.
3. **Extractives** (water soluble) [C, H, O, N, S].  
*Hypoxanthin*:  $C_5H_4N_4O$  (Thudichum<sup>5</sup>). It seems curious that Thudichum should mention this as the only purin base found, when Levene gives, as the purin bases of his nucleoproteid, adenin and guanin, and states that hypoxanthin was not present.

<sup>1</sup> HALLIBURTON: Collected papers from the Physiological Laboratory, of King's College, London, 1893, No. 1.

<sup>2</sup> SCHKARIN: Inaugural Dissertation, St. Petersburg, 1902.

<sup>3</sup> LEVENE: Archives of neurology and psychopathology, 1899, v, II. p. 1.

<sup>4</sup> KÜHNE and CHITTENDEN: Zeitschrift für Biologie, 1890, xxvi, p. 291.

<sup>5</sup> THUDICHUM, J. L. W.: Die chemische Konstitution des Gehirns des Menschen und der Tiere, 1901, F. Pietzcker, Tübingen, p. 319.



isolated as a mixture of isomers and homologues by Thudichum<sup>1</sup> and Koch<sup>2</sup> are all characterized by the presence of three methyl groups attached to nitrogen. One methyl group splits off quantitatively at 240° C. with hydriodic acid, the remaining two split off at 300° C. These lecithins have basic properties, form double salts with cadmium chloride, but do not form insoluble lead salts. They are soluble in alcohol and ether. Phosphorus, 4 per cent; nitrogen, 1.8 per cent. Proportion of 1 : 1.

*Amidolecithins*: Amidomyelin,  $C_{44}H_{88}N_2PO_8$ . Isolated so far only by Thudichum,<sup>3</sup> who has not published complete analyses. From the empirical formula, this substance evidently is closely allied to the lecithins, and should have three methyls attached to nitrogen. The quantitative results indicate that this substance can be present only in extremely small amount. Nitrogen, 3.5 per cent; phosphorus to nitrogen as 1 : 2.

*Kephalins*: Kephalin,  $C_{42}H_{79}NPO_{13}$  (Thudichum,<sup>4</sup> Koch<sup>5</sup>);  
Oxykephalin,  $C_{42}H_{79}NPO_{14}$  (Thudichum<sup>6</sup>);  
Peroxykephalin,  $C_{42}H_{79}NPO_{15}$  (Thudichum<sup>7</sup>);  
Myelin,  $C_{40}H_{75}NPO_{10}$  (Thudichum<sup>8</sup>).

These substances are derived from the lecithins by the loss of two methyl groups and an oxidation of the oleic acid radicle, the chemistry of which is still very obscure. In consequence of the nitrogen becoming a triad, the basic properties are lost and these substances consequently give insoluble lead salts. The methyl group is split off quantitatively at 240° C. with hydriodic acid. No more methyl is split off above that temperature. A comparison of the formula makes it evident that kephalin  $C_{42}$  . . . is derived from stearyloley lecithin  $C_{44}$  . . . and myelin  $C_{40}$  . . . from palmityloley lecithin  $C_{42}$  . . . These substances are soluble in ether, insoluble in alcohol. Phosphorus, 3.7 per cent; nitrogen, 1.7 per cent. Proportion of 1 : 1.

*Amidokephalins*: Amidokephalin,  $C_{42}H_{80}N_2PO_{13}$ . Isolated so far only by Thudichum,<sup>9</sup> may be considered to be derived from the amidomyelin by the loss of two methyl groups and an oxidation similar to kephalin. The empirical formulæ support this theory. Phosphorus to nitrogen as 1 : 2.

<sup>1</sup> THUDICHUM: *Loc. cit.*, p. 322, 123, 110.

<sup>2</sup> KOCH, W.: *Zeitschrift für physiologische Chemie*, 1902, xxxvi, p. 134.

<sup>3</sup> THUDICHUM: *Loc. cit.*, p. 322, 123, 110.

<sup>4</sup> THUDICHUM: *Loc. cit.*, p. 320, 130.

<sup>5</sup> KOCH: *Loc. cit.*

<sup>6</sup> THUDICHUM: *Loc. cit.*, p. 138.

<sup>7</sup> THUDICHUM: *Loc. cit.*, p. 138.

<sup>8</sup> THUDICHUM: *Loc. cit.*, pp. 323, 156.

<sup>9</sup> THUDICHUM: *Loc. cit.*, p. 107.

Besides the above-mentioned, there have been isolated by Thudichum, but not so far confirmed by me and difficult to classify, *paramyelin*,  $C_{38}H_{75}NPO_9$ , probably related to lecithin, *sphingomyelin*,  $C_{52}H_{104}N_2PO_9$ , probably related to the amidolecithins [nitrogen, 3 per cent], containing however no glycerine and *assurin*,  $C_{46}H_{94}N_2P_2O_9$ . [Phosphorus, 7 per cent; nitrogen, 3.2 per cent.]

#### 6. Cerebrins [C, H, O, N].

Within recent years these substances have been studied on human material by Thudichum<sup>1</sup> and Thierfelder,<sup>2</sup> on sheep's brains by Koch,<sup>3</sup> and on horse's brains by Bethe.<sup>4</sup> As there is very little difference in the cerebrins derived from different species it will simplify matters to compare them regardless of their source.

*Phrenosin*,  $C_{41}H_{79}NO_8$ , Thudichum<sup>5</sup> may be said to be identical or isomeric with the substance isolated by Thierfelder and by Koch, as will be seen from a comparison of the analyses.

	Thudichum.	Thierfelder.	Koch.
Carbon . . . .	69.00	69.16	68.73
Hydrogen . . . .	11.08	11.54	11.83
Nitrogen . . . .	1.96	1.76	1.64

Phrenosin splits off galactose on heating with dilute mineral acids.

*Kerasin*,  $C_{44}H_{88}NO_8$  (Thudichum<sup>6</sup>), probably identical with Bethe's<sup>7</sup> *amidocerebrin acid glycosid*,  $C_{44}H_{81}NO_8$ . Both are undoubtedly homologues of phrenosin and like it split off galactose.

*Phrenin*, isolated by Bethe, was obtained by myself as a decomposition product from cerebrin after boiling with dilute hydrochloric acid. The analyses agree very well.

	Bethe. <sup>7</sup>	Koch.
Carbon . . . .	71.90	71.60
Hydrogen . . . .	11.95	12.14
Nitrogen . . . .	1.5	1.89

This substance does not split off a reducing sugar as do phrenosin and kerasin.

<sup>1</sup> THUDICHUM: *Loc. cit.*, p. 178.

<sup>2</sup> THIERFELDER. H.: *Zeitschrift für physiologische Chemie*, 1900, xxx, p. 549.

<sup>3</sup> KOCH: *Loc. cit.*

<sup>4</sup> BETHE, A.: *Archiv für experimentelle Pharmakologie und Pathologie*, 1902, xlviii, p. 78.

<sup>5</sup> THUDICHUM: *Loc. cit.*, p. 184.

<sup>6</sup> THUDICHUM: *Loc. cit.*, p. 218.

<sup>7</sup> BETHE: *Loc. cit.*, p. 184.



*Cerebrin acids* so far only isolated by Thudichum.<sup>1</sup>

*Cerebrin acid*,  $C_{49}H_{99}NO_{11}$ , and *sphaerocerebrin*,  $C_{58}H_{123}NO_{17}$  are characterized by forming lead salts insoluble in hot alcohol. This distinguishes them from kersin and phrenosin, which do not combine with lead. Cerebrin acid, according to Thudichum, splits off galactose. A comparison of the percentage of carbon found by Thudichum makes it extremely probable that these substances are intermediary oxidation products of phrenosin and kersin, as indicated by the following figures :

	Phrenosin.	Cerebrin acid.	Sphaerocerebrin.
Carbon . . . . .	69.0	67.00	62.75
Hydrogen . . . . .	11.08	11.36	11.08
Nitrogen . . . . .	1.96	1.59	1.23

The large number of carbon atoms given by Thudichum in the empirical formula is based on the nitrogen determination, which is not strictly correct.

No other substances have been isolated which may be said to belong to the group of the cerebrins. The cerebrinphosphoric acid of Bethe is only an impure mixture which undoubtedly contained sulphur, for which he neglected to test. None of the members of this group can be said to be pure until they are free from sulphur and phosphorus, both of which elements cling to them most tenaciously. All the cerebrins, when pure, are insoluble in ether and soluble in hot alcohol. The cerebrin acids are more soluble in glacial acetic acid than phrenosin.

7. **Cholesterin**  $[C, H, O]$ ,  $C_{27}H_{45}OH$ , said to occur in the brain as free cholesterin and in the form of esters. Soluble in hot alcohol and cold ether.
8. **Sulphur compounds**  $[C, H, O, N, P, S]$ .

Any extract of brain tissue with alcohol and ether will be found to contain considerable amounts of sulphur. None of this sulphur is, however, present as cystein sulphur, as saponification of the extract with sodium hydrate, and subsequent treatment with lead acetate will give no lead sulphide. Thudichum<sup>2</sup> has isolated an impure mixture containing a large amount of sulphur [4 per cent] and also some phosphorus, mixed with some of the cerebrin acids. On this account, he is inclined to look upon them as cerebro sulphatids. Any ordinary preparation of cerebrin will contain sulphur, and one might be inclined to look upon the sulphur as a constituent element. I have been able, however, by means of solvents to obtain, on the one hand, a cerebrin free from sulphur, and on the other

<sup>1</sup> THUDICHUM: *Loc. cit.*, p. 221.

<sup>2</sup> THUDICHUM: *Loc. cit.*, p. 224.

hand, a compound containing up to 4 per cent of sulphur and free from any reducing sugar. The preparation also contains about 2 per cent of phosphorus, 5 per cent of nitrogen, and some fatty acids. It is undoubtedly still impure. The substance obtained from sheep's brains is so difficult to manipulate that I will have to use for this work human brains, which yield a much more tractable preparation. As the collection of sufficient material for this investigation will of necessity be rather slow, I should like to reserve the study of this interesting sulphur compound a little longer, and will describe the methods of preparation and give analyses when I have been able to obtain the substance in larger quantity. The largest amount obtained so far was 0.3 grams and that was impure.

9. **Amidofats** [C, H, N, O].

*Krinosin*,  $C_{38}H_{79}NO_6$ .

*Bregenin*,  $C_{40}H_{81}NO_5$ .

These substances have so far been only isolated by Thudichum.<sup>1</sup> They are distinguished from fats by their insolubility in ether. The quantitative determinations indicate that they may be present in small amount only. They most probably represent post-mortem decomposition products.

10. **Monophosphatids** [C, H, O, P].

*Lipophosphoric acid*, *butophosphoric acid*. Isolated by Thudichum,<sup>2</sup> but not analyzed completely. Contain about 4 per cent of phosphorus, and are free from nitrogen. May be present in slight amount in white matter, as not quite all the phosphorus is accounted for. Most probably, however, they are either post-mortem decomposition products or the result of chemical manipulations.

This completes the list of substances isolated or supposed to have been isolated from brain tissues. Free fats and fatty acids have never been found to be present in normal brain tissue. Bethe<sup>3</sup> mentions stearic acid, but adds a question mark, which is a wise provision, as he has been rather unfortunate in describing decomposition products as primary constituents (phrenin, see above). Protagon, I will not attempt to resurrect, as the work of Thierfelder<sup>4</sup> and of Gies<sup>5</sup> has settled its fate.

<sup>1</sup> THUDICHUM: *Loc. cit.*, p. 227.

<sup>2</sup> THUDICHUM: *Loc. cit.*, p. 177.

<sup>3</sup> BETHE: *Loc. cit.*, p. 86.

<sup>4</sup> THIERFELDER: *Loc. cit.*, p. 540.

<sup>5</sup> LESEM, W. W., and GIES, W. J.: This journal, 1902, viii, p. 183.

#### DETERMINATION OF WATER.

In devising a method for the determination of the amount of water in a tissue, it is necessary to consider the colloidal nature of the substances present. In every tissue there may be said to be a tendency towards an equilibrium between the water existing in the free state and that imbibed by the colloid, on the one hand, and the water imbibed and the water of constitution, on the other hand. In other words, water of constitution cannot become free without passing through the intermediary state of imbibed water. This equilibrium is subject to considerable variations, depending on temperature and pressure, and to a large extent also on the presence of inorganic salts. The simplest method of removing the water from such a system would be to raise the temperature, which would cause the imbibed water to become free and evaporate. The combined water would also evaporate, but only towards the end. The objection to this method lies in the fact that the colloids coagulate, as the temperature is raised, and form a firm crust at the surface, through which even the free water can only evaporate with difficulty. Another method, which indeed would be the ideal one, would be to extract the water with some solvent, like absolute alcohol, which, however, must not dissolve anything else, — a condition that no solvent fulfils. The only method which remains, therefore, is to allow the water to evaporate at as high a temperature as possible, but below the point at which the colloids coagulate ( $40-42^{\circ}\text{C.}$ ). As such a process would be extremely slow, it must be hastened by a good vacuum pump. Even in a vacuum, however, as the space surrounding the tissue becomes filled with water vapor, there is a tendency for the water to go back into the tissue, probably in the state of imbibed water. The presence of a good drying agent, like calcium chloride, is, therefore, a necessity, and greatly accelerates the process. The advantage of this method of drying lies in the fact that as the surface becomes dry, it can take up moisture from the lower layers on account of its ability to imbibe water. The tissue, therefore, becomes uniformly dried throughout, and constant weight is reached in a remarkably short time. The drying should, however, extend over at least a week, to be thorough. Between each weighing there should be a period of drying in a constant vacuum for at least twenty-four hours. To test the efficiency of this method in affecting a complete drying, two samples of cortical

grey matter, and one sample of corpus callosum were first dried between weighed watchglasses, fitted with a clip, to constant weight at 40° C. and then heated to 95°–100° C. The copper vacuum oven employed, was surrounded with a water jacket and was capable of holding a vacuum for at least two days.

	I grey.	II white	III grey.
Weight of material as collected . . . . .	0.7761	1.6180	0.8976
Weight after 36 hours drying at 40° C.	0.1365	0.4917	0.1624
Weight after 60 hours drying at 40° C.	0.1340	0.4837	0.1591
Weight after 84 hours drying at 40° C.	0.1350	0.4831	0.1599
Weight after 108 hours drying at 40° C.	0.1340	0.4803	0.1585
Weight after 132 hours drying at 40° C.	0.1340	0.4803	0.1585
Weight after 18 hours drying at 95° C.	0.1336	0.4801	0.1583
Per cent of water at 40° C. . . . .	82.74	70.31	82.34
Per cent of water at 95° C. . . . .	0.05	0.01	0.02

This method gives, therefore, an accurate determination of the amount of water present in the tissues as free and imbibed water under the conditions of temperature and concentration of salts at which the tissues happen to be at the time of the experiment. The presence of fats, by preventing the free evaporation of the water, interferes very much with the determination and makes it much more tedious. It is well after the first weighing to loosen the tissues from the watch-glass (to which they usually stick rather firmly) in order to expose a larger surface for evaporation. The determination of water is of no value in establishing differences between normal and pathological material, as too many factors enter in and cause considerable variations. These factors are:

1. The amount of blood in the tissue at death, whether congested or anæmic.
2. The accidental and almost incontrollable variations in the amount of drying during the collection of the material.
3. In the case of cortical grey matter the varying amounts of white matter introduced in the process of collection or actually present as fibres in the cortex. This error can, however be determined and probably eliminated.

In spite of these objections, it is, however, necessary to determine the amount of water in an investigation of this kind, in which every constituent is to be determined with a view to adding the sum total up to 100 per cent.

DETERMINATION OF PROTEIDS (INCLUDING THE EXTRACTION OF  
THE OTHER CONSTITUENTS).

A consideration of the constituents of nerve tissues mentioned before shows that they all dissolve in either alcohol or ether or both, with the exception of the proteids, some of the inorganic salts, and possibly the small amount of albumoses and peptones that may be present. The various proteids are distinguished from one another as follows: The neurokeratin is insoluble in ferments and sodium hydrate; the nucleoprotein is characterized by its phosphorus; and the simple proteids can be determined by difference. The extraction of the alcohol-ether-soluble and the water-soluble constituents is carried on as follows:

5 to 10 gm. of the substance after weighing are extracted first with hot alcohol (just below the boiling point) for six hours, filtered hot and washed several times with hot alcohol. The filtering is done through a filter paper which has been tared against another filter paper, so that small adhering quantities of insoluble matter may not be lost in the final weighing of the proteids. The insoluble residue is then allowed to stand in a flask with cold ether over night. The hot alcoholic filtrate deposits on cooling a number of substances which would be immaterial, if only one determination were to be made. In the complete determination of all the constituents of nerve material, however, this would multiply the number of extractions to an inconvenient amount, and it is preferable to introduce a slight error by making up the warm filtrate to 110 c.c. in a graduated flask with warm alcohol, and dividing it into four portions with a warmed 25 c.c. pipette. This gives four determinations to one extraction, which greatly simplifies the work. The extra 10 c.c. left in the flask must be corrected for in the final calculation, and go to correct slight sources of error, such as the amount of solution adhering to the sides of the flask or lost in the transferring. The ether extraction, after filtering, is distributed in the same manner, but must not be mixed with the alcohol in order to save solvent in making up to the mark until after the third ether extraction, as otherwise an insoluble precipitate is formed, which cannot be distributed with any

degree of accuracy. The alternate extraction with hot alcohol during the day and cold ether during the night is continued for ten days. After the third ether extraction, it is well, however, to transfer the material to a mortar and pound it into fine shreds with a pestle. It is astonishing to see how tissues which at first are so difficult to obtain in a fine state of division will break up under this treatment into the finest fragments, which can be very thoroughly extracted. After ten days' extraction, it is needless to continue the ether extraction, but the alcohol should be continued at least five days longer, in order to remove the last traces of lecithin from the now coagulated proteid.

All the insoluble residue is transferred to the tared filter paper, and dried to constant weight in a vacuum oven at 40° C. The residue is then moistened with chloroform water, and extracted several times to remove the remaining inorganic salts and organic extractives. The amount extracted is determined as described under the determination of the extractives, and subtracted from the above weighing. The result represents the *total proteids*. The *neurokeratin* is then determined according to the method of Kühne and Chittenden<sup>1</sup> in one sample. In another sample the phosphorus is determined (after destruction of the organic matter) by the molybdate method, and the approximate amount of Levene's *nucleoproteid* determined by multiplying the *phosphorus* found, by the factor 175.4. The remainder, after subtracting the nucleoproteid and neurokeratin from the *total proteids*, represents the *simple proteids* or *globulins* of Halliburton. It would be desirable further to check the determination of Levene's nucleoproteid by an estimation of the nuclein bases. These are present, however, in such small amount, and the present methods contain so many sources of error, that no reliance could be placed on such a result.

#### THE DETERMINATION OF EXTRACTIVES (WATER SOLUBLE) AND INORGANIC SALTS.

The usual method of determining the extractives and inorganic salts by extraction with water and removal of the proteids by boiling in slightly acid solution cannot be employed in the case of brain tissues on account of the large proportion of lecithans present. The lecithans go into colloidal solution and cannot be removed by boiling. In slightly acid solution (0.32 HCl) the lecithans can, however, very easily be precipitated by addition of a little chloroform. The solu-

<sup>1</sup> KÜHNE and CHITTENDEN: *Loc. cit.*, p. 295.

tion, at first opalescent, becomes cloudy, and in the course of a day the precipitate settles out leaving a clear solution which can then be decanted off. Unfortunately, the proteids are not removed by this method, and it becomes necessary to harden or render them insoluble, first by long treatment with alcohol. As this hardening is best combined with the usual extraction described before, the following method has been found to yield the best results. As the largest part of the extractives and inorganic salts is found in the ether-alcohol soluble portion their determination will be described first.

**A. Determination of the extractives and inorganic salts in alcohol-ether-soluble portion.** — All or a known portion of the alcohol-ether extract is carefully evaporated to a small bulk on the water bath and the remaining liquid removed in a vacuum desiccation over calcium chloride. This avoids any danger of breaking up the larger molecules by heating to a high temperature. The residue is emulsified with 40 c.c. of water, and transferred to a graduated 100 c.c. flask. 1 c.c. of concentrated hydrochloric acid, and fifteen drops of chloroform are added, the mixture thoroughly shaken and made up to the mark. After settling, which takes longer with grey than with white matter, the now perfectly clear solution is filtered through a dry filter paper and 80 c.c. evaporated to dryness on the water bath in a platinum dish, dried at  $105^{\circ}$  C., and *weighed*. (Total extractives and inorganic salts.) The residue should again dissolve easily in water to form a clear solution, which should give no cloudiness on shaking with chloroform water. This residue can then be used for the determination of the inorganic sulphates, phosphates, the extractive nitrogen, or the total inorganic salts.

**Inorganic salts.** — The residue is heated to a dull red heat, not high enough to volatilize chlorides, and still sufficiently high to burn all the carbon and leave a pure white ash. The weight of *inorganic salts* is then subtracted from the weighing obtained above, and the difference represents the *organic extractives*.

The sources of error in this method appear to be *first*, the danger of splitting up larger molecules (cerebrin) with the hydrochloric acid. This may be reduced to a minimum by keeping the solution fairly cool, and not leaving the acid for too long a time in contact with the colloids. *Second*, the possibility of some of the extractives dissolving in the chloroform. This source of error may be reduced to a minimum by using only a small amount of chloroform, and also by counting in its volume in making up the solution to the mark.

The error due to the action of the acid is very slight, as will be seen from the following figures:

g. 2899 gm. of corpus callosum were extracted with alcohol and ether, and the extractions divided into four portions. Two portions were used for this determination. One was allowed to stand with the acid one day, the other seven days. They were made up to 100 c.c. and 80 c.c. used for the evaporation.

I. 1 day.		II. 7 days.	
Residue on evaporation	34.1 mgm.		33.1 mgm.
Per cent of total	1.84		1.78

The one exposed to the acid for a longer time contained even less than the other, the variation is, however, within the limit of error.

b. **Determination of the extractives and inorganic salts in the alcohol-ether-insoluble portion.**—All of the extractives and inorganic salts do not dissolve in the alcohol and ether. It is necessary to extract the residue (left after the last alcohol extraction: see proteid determination) with water to which a little chloroform has been added. About three or four extractions are sufficient, the solution is evaporated to dryness in a platinum dish, dried and weighed and the inorganic salts determined by ignition. The organic extractives are, as before, represented by the difference. The following figures will give an idea of the relative amount of material to be found in the two portions:

	Total residue.	Residue on ignition.	Per cent.	
			Organic.	Inorganic.
Alcohol-ether-soluble portion	165.6 mgm.	57.6 mgm.	1.16	0.62
Alcohol-ether-insoluble portion	50.2 mgm.	18.6 mgm.	0.35	0.20
			1.51	0.82

The value obtained for the inorganic salts by this method is especially satisfactory, as it comes very near the amount required of an isotonic salt solution. The organic extractives are given as a whole, the determination of the individual constituents will have to be preceded by a more careful qualitative study. At present very little is known of this important group.

#### DETERMINATION OF THE LECITHANS.

The members of this group, as far as investigated, have all been found to be characterized by one or more methyl groups attached to nitrogen. Cerebrins and the sulphur compound do not contain such



a group. Among the extractives there may at times be present, neuridin, cholin, and other substances which contain methyl attached to nitrogen. In applying Herzig and Meyer's method of determining the methyls attached to nitrogen, to the estimation of the lecithans, in the case of tissues like the brain, it is always necessary, therefore, to determine the amount of methyl groups present in the extractives by a separate analysis, and apply a correction. Although this correction is hardly ever very great, calculated as cholin, when calculated as lecithin, on account of the higher molecular weight, it may amount to as much as 0.5 per cent. The members of this group contain either one or three methyl groups. No substance with two methyl groups has so far been found. To distinguish between the members of this group, the temperatures at which the methyls split off are made use of. The following gives the classification in a tabulated form:

Split off one $\text{CH}_3$ below $240^\circ \text{C.}$ ; none above $240^\circ \text{C.}$	Split off one $\text{CH}_3$ below $240^\circ \text{C.}$ ; two $\text{CH}_3$ below $300^\circ \text{C.}$ , and above $240^\circ \text{C.}$
Kephalin	Stearyloley l lecithin
(Oxykephalin)	Margeryloley l lecithin
(Peroxykephalin)	
Myelin	Palmityloley l lecithin

The amidomyelin, amidokephalin, sphingomyelin, and assurin have not been investigated with reference to their methyl groups. As they contain a higher percentage of nitrogen than the other members of this group, some idea as to their quantity may be arrived at from a consideration of the difference between the *nitrogen calculated* present as lecithin, myelin, kephalin, cerebrin, sulphur compound and extractive on the one hand, and the *total nitrogen found* in the ether-alcohol extract on the other hand. As this difference falls within the limits of error of the determination, these so-called amido lecithans can be present only as traces. Their high percentage of nitrogen makes it indeed probable that they may be ordinary lecithin or kephalin mixed with some of the sulphur compound. It is quite as difficult to separate this compound from the lecithans as from the cerebrin. Thudichum does not mention testing his preparations for sulphur. The existence of paramyelin I have not been able to confirm, and it may, therefore, be neglected.

**Determination of lecithins and kephalins:** All or a definite part of the alcohol-ether extract is evaporated in a dish or in the double bulb be-

longing to the apparatus previously described by me.<sup>1</sup> On account of the small size of the double bulb, the evaporation of the always considerable amounts of alcohol and ether is very laborious, and the determination is often lost on account of the tendency of the ether to superheat and as a result boil over. It is more convenient, therefore, to evaporate in an open dish to small bulk, and dry in vacuum until most of the alcohol is removed. The residue is then transferred to the bulb by dissolving as much as possible in ether, and transferring the insoluble portion mechanically, taking great care, however, not to lose *anything*. The ether is then evaporated by gentle heating, and the residue dried in a stream of dry air at about 50° C. to remove every *last trace of alcohol*. Slight amounts of moisture introduce no serious error.

After the residue is thoroughly freed from alcohol it is heated in the apparatus previously mentioned, with ammonium iodide and hydriodic acid (sp. gr. 1.5) to a temperature not exceeding 240° C. As the thermometer is placed in a sand bath, this temperature is not absolute and may be subject to considerable variation. (The bulb of the thermometer should be about even with the bottom of the double bulb.) Usually the alcoholic silver nitrate solution in which the methyl iodide is converted into silver iodide begins to become cloudy when a temperature of 160° C. is reached in the sand bath. If the temperature is not raised too suddenly, a point is reached, after twenty minutes or so, at which the silver nitrate solution becomes perfectly clear, and the precipitate has all settled to the bottom. With the experiment properly adjusted, the temperature of 240° C. should be reached at just about the time at which the solution becomes clear. If the silver nitrate solution has not become clear, the temperature should be kept below 240° C. until it does. The precipitate of silver iodide and the supernatant excess of silver nitrate are then removed, and represent all the methyl from kephalin and myelin and one group from the lecithins. Another flask, containing silver nitrate solution, is now attached, and the temperature allowed to rise to 300° C. The liquid should remain clear for a considerable time, as there is quite a range of temperature between the point at which the first and the last two methyls split off. Usually at 280° C. the second portion of the methyl iodide begins to come over as indicated by the silver nitrate solution becoming cloudy. When a tem-

<sup>1</sup> Koch, W.: University of Chicago, Decennial Publications, 1902, x, p. 95.

perature of 320° C. is reached, the liquid is again clear, and the determination at an end. The two portions of silver iodide are poured into twice to three times their bulk of water, first freed from alcohol by heating on the water bath and then acidified with nitric acid, filtered through a Gooch crucible, washed, dried, and weighed. The amount of silver iodide obtained at 300° C. (minus the amount obtained for the extractives in a separate determination), multiplied by the factor 1.661, gives the amount of lecithins (assuming 4 per cent phosphorus and 1.8 per cent nitrogen). The amount of silver iodide obtained below 240° C. (minus the amount obtained for the extractives in a separate determination), multiplied by the factor 3.325, gives the total amount of lecithins, kephalin, and myelin. The amount of lecithins found previously must then be subtracted from this total, and the remainder represents approximately the kephalin and myelin. To obtain their exact value, this result must be divided by the factor 0.925, which corrects for the relatively smaller amount of phosphorus found in kephalin, and its larger molecular weight. To distinguish between kephalin and myelin would be of no more value than to distinguish between stearyl-oleyl lecithin and palmityl-oleyl lecithin, as they stand in the same relation to one another, being homologues. The following may serve as an illustration of the methods of calculation described above :

10.0661 gm. of cortical grey matter were extracted with alcohol and ether, and the extractions divided into five portions. One was used to determine the *total methyl*; in another the extractive methyl was determined; the remainder were used for other determinations.

Silver iodide below 240° C. . . . .	25.1 mgm. AgI
Correction for CH <sub>3</sub> in extractives . . .	2.0 mgm. AgI
	<hr/>
	23.1 × 3.325 = 76.81 mgm. total.
Silver iodide at 300° C. . . . .	39.6 AgI
Correction for CH <sub>3</sub> in extractives . . .	1.6 AgI
	<hr/>
	38.0 × 1.661 = 63.12 mgm. lecithin.
	0.925 ÷ 13.69 = 14.80 mgm. <sup>kephalin.</sup> myelin.

In per cent of total: 3.14 per cent lecithins; 0.74 per cent kephalin and myelin.

## VI. DETERMINATION OF THE CEREBRINS.

The members of this group are all characterized by splitting off galactose with dilute hydrochloric acid. Phrenin need not be con-

sidered as it is a decomposition product of phrenosin. The cerebrin acids give lead salts insoluble in hot alcohol, which distinguishes them from phrenosin and kersin. No other substances found in the alcohol-ether-soluble portion of brain-tissues contain a reducing sugar as a part of their molecule. There may occasionally be some sugar present in the extractives, but this error can be eliminated. The method of determining these substances by the amount of copper sulphate reduced in alkaline solution by the sugar split off with dilute acid, has already been made use of by Noll<sup>1</sup> for the estimation of protagon. As his analyses have been made carefully, it is merely necessary to recalculate them by multiplying the amount of copper reduced with the correct factor. The method of titrating the copper with potassium cyanide employed by him is not sufficiently accurate for the small amounts of cerebrin occasionally found under pathological conditions, especially in the cortex. It is preferable therefore to filter the reduced cuprous oxide through a Gooch crucible, and oxidize to copper oxide by heating to a red heat for half an hour. The danger of incomplete oxidation is very slight with the small amounts of copper to be considered in these analyses (less than 100 mgm.).

**Determination of copper value of phrenosin (cerebron).**— The sample of phrenosin (cerebron) was prepared from brains of the insane, and carefully purified by recrystallization from glacial acetic acid and alcohol until it was free from phosphorus and contained less than 0.07 per cent of sulphur. The sample was also free from cerebrin acids, as it gave no precipitate with lead acetate in alcohol solution. Whether or not a substance prepared from brains of the insane is of value for the comparison of normal and pathological brains cannot be decided off hand. A comparison will have to be made with a preparation from normal brains as soon as sufficient material can be obtained. The phrenosin was dried in vacuo, 1 gm. weighed out and split up with 150 c.c. of 1 per cent hydrochloric acid by heating over a free flame to gentle boiling under a return condenser for twenty hours.<sup>2</sup> The results obtained were plotted as copper oxide and phrenosin, and from the plot the following table arranged:

<sup>1</sup> NOLL, A.: *Zeitschrift für physiologische Chemie*, 1899, xxvii, 370.

<sup>2</sup> The solution was made up to 250 c.c., and the copper reduced determined for the following quantities: 50 c.c.; 40 c.c.; 35 c.c.; 30 c.c.; 25 c.c.; 20 c.c.; 15 c.c.

Table giving the amount of copper oxide (CuO) corresponding to a given quantity of phrenosin (cerebron), (also essentially the same for kersin, cerebrin, and cerebrin acids).

CuO.	Phrenosin.	CuO.	Phrenosin.	CuO.	Phrenosin.	CuO.	Phrenosin.
milligrams	milligrams	milligrams	milligrams	milligrams	milligrams	milligrams	milligrams
6	18.4	27	65.9	48	113.6	69	160.9
7	20.7	28	68.2	49	115.8	70	163.2
8	23.0	29	69.5	50	118.0	71	165.5
9	25.3	30	72.8	51	120.2	72	167.8
10	27.6	31	75.1	52	122.5	73	170.1
11	29.9	32	77.4	53	124.7	74	172.4
12	32.2	33	79.6	54	127.0	75	174.7
13	34.4	34	81.9	55	129.2	76	177.0
14	36.7	35	84.1	56	131.5	77	179.3
15	38.9	36	86.4	57	133.7	78	181.5
16	41.2	37	88.6	58	136.0	79	183.8
17	43.4	38	90.8	59	138.2	80	186.1
18	45.7	39	93.1	60	140.5	81	188.4
19	47.9	40	95.4	61	142.7	82	190.7
20	50.2	41	97.6	62	145.0	83	192.9
21	52.4	42	99.9	63	147.3	84	195.2
22	54.7	43	102.3	64	149.6	85	197.5
23	56.9	44	104.6	65	151.8	86	199.8
24	59.2	45	106.8	66	154.0	87	202.1
25	61.4	46	109.1	67	156.3	88	204.4
26	63.7	47	111.3	68	158.6	89	206.7

a. **Determination of total cerebrins.** — All or a known portion of the alcohol-ether extract of a weighed quantity of brain tissue is evaporated to small bulk on a water bath and completely freed from alcohol in a vacuum desiccator. The residue is emulsified with water, transferred to a graduated flask, and hydrochloric acid and chloroform added, as described in the determination of the extractives. After the precipitate has settled, the liquid is filtered off and any sugar which may be present in the free state thus removed. The

residue is dissolved in hot alcohol, transferred to a 200 c.c. flask, the alcohol evaporated and the residue heated to gentle boiling for twenty hours with 1 per cent hydrochloric acid under a return condenser over a free flame. The solution is transferred to a graduated flask, and enough saturated solution of sodium sulphate added to obtain a clear and rapid filtration. An aliquot part of the clear filtrate is neutralized with sodium hydrate and 10–20 c.c. Fehling's solution added in the cold. The solution, if free from precipitate, is warmed on a water bath, heated to boiling over a free flame, and again allowed to stand on a water bath for several hours. This insures the formation of a coarse-grained brick-red precipitate which filters very easily. The liquid over the precipitate must be of a deep blue color and not a pale greenish blue, otherwise the reduction has not been properly made. The concentration of the sugar in the solution should never exceed 0.2 per cent. The precipitate is filtered through a Gooch crucible, washed thoroughly with hot water, dried, ignited, and weighed. (*Total cerebrins*.)

b. **Determination of cerebrin acids.** — Another portion of the alcohol-ether extract is freed from ether by gentle warming, to the warm alcohol solution a little ammonia water added, and then an excess of alcoholic lead acetate. The precipitate is transferred to a large porcelain Gooch crucible, and washed with hot alcohol until nothing more dissolves. From the filtrate a precipitate separates out on cooling, which does not contain any cerebrin acids. The precipitate in the Gooch crucible is treated with dilute hydrochloric acid as described before for *total cerebrins*. The amount of cerebrin acids usually found is so small that there is no serious error in calculating them from the table given for phrenosin. The amount of copper oxide found is subtracted from the copper oxide found for total cerebrins and the amount of phrenosin corresponding to the remaining copper oxide read off from the table. A comparison of phrenosin as determined by me, and a result of Noll recalculated, gives the following very interesting agreement:

	Noll <sup>1</sup> (protagon, 6.33).	Koch.
Per cent of phrenosin in white matter . . . . .	4.67 per cent	4.57

Noll's preparation of protagon contained about 75 per cent of cerebrin, the remainder consisted of sulphur compound and either lecithin or more probably kephalin.

<sup>1</sup> NOLL, A.: *Loc. cit.*

#### THE DETERMINATION OF CHOLESTERIN.

The cholesterin is best determined according to the very excellent method of Ritter.<sup>1</sup> All or a known portion of the alcohol-ether extract is saponified with sodium hydrate, the alcohol evaporated, the excess of alkali almost neutralized with hydrochloric acid, evaporated to dryness, and sufficient sodium chloride added to permit the grinding up of the slightly alkaline mass in a mortar. The perfectly dry powder is extracted with ether, the ether evaporated, and the residue again dissolved in perfectly dry ether, and evaporated. The glycerine is left behind in this manner. The residue is dried at 105 C. and weighed. A comparison of an analysis by Baumstarck (quoted by Hammarsten) with my analysis shows a fair agreement. This method has a tendency to give a better yield of cholesterin than any so far devised.

	Baumstarck. <sup>2</sup>	Koch.
Per cent of cholesterin in white matter . . . . .	4.52	4.86

#### DETERMINATION OF SULPHUR COMPOUND.

In the absence of more definite knowledge of the chemical structure of the sulphur compound, it must be determined by the amount of sulphur found. As inorganic sulphates are always present and interfere with this determination it is necessary to treat the alcohol-ether extract as described for extractives. The sulphur in the solution and in the precipitate is then determined. The sulphur found in the precipitate multiplied by 25 gives the approximate amount of sulphur compound. This assumes that the pure compound has 4 per cent of sulphur, which may be too low. For purposes of calculation, the phosphorus may be assumed to be 2 per cent and the nitrogen 5 per cent.

#### AMIDO FATS AND MONOPHOSPHATIDS.

There is at present no method of determining these substances directly. They most probably represent post-mortem decomposition products, and can be present only in very small quantity, as the amount of phosphorus and nitrogen not accounted for in terms of other constituents is within the limits of error. The existence or non-existence of these substances in normal, unchanged nerve tissue

<sup>1</sup> RITTER, E.: *Zeitschrift für physiologische Chemie*, 1901, xxxiv, p. 456.

<sup>2</sup> HAMMARSTEN, O.: *Lehrbuch der physiologischen Chemie*, 1899, p. 371.

must, therefore, be decided with larger amounts of material. In nerve tissues undergoing active degeneration, they may be present in larger amount.

#### SUMMARY OF ANALYSES.

Of the analyses so far made with the methods outlined above, I will give two, with the reservation, however, that they be regarded as preliminary and merely illustrative of the methods. The determination of the composition of the healthy human brain will require a large number of analyses.

The material with which the following two analyses were made was obtained from an epileptic who had died during a seizure. The post-mortem was made twenty-four hours after death, and the material immediately collected and analyzed.

#### CHEMICAL COMPOSITION OF HUMAN BRAIN (EPILEPTIC).

	Corpus callosum.	Cortex (prefrontal)
Water . . . . .	67.97	84.15
Simple proteids . . . . .	3.2 (by difference)	5.0 (by difference)
Nucleoproteid . . . . .	3.7	3.0
Neurokeratin . . . . .	2.7 (Chittenden)	0.4 (Chittenden)
Extractives . . . . .	1.51	1.58
Inorganic salts . . . . .	0.82	0.87
Lecithins . . . . .	5.19	3.14
Kephalin and myelin . . . . .	3.49	0.74
Amido lecithans . . . . .	trace	trace
Phrenosin and kersin . . . . .	4.57	1.55
Cerebrin acids . . . . .	trace	none
Cholesterin . . . . .	4.86	0.7
Sulphur compound . . . . .	1.40	1.45
	<u>99.41</u>	<u>102.58</u>

The total for the corpus callosum indicates that pretty nearly everything has been accounted for. The rather high result of the grey matter is probably due to the greater difficulty in obtaining a uniform sample. Such a number of analyses could not possibly be made on one sample. The distribution of the nitrogen and phosphorus and comparison with the total found indicates that the most important constituents of the alcohol-ether soluble portion at least have been accounted for, as will appear from the following tables.



PHOSPHORUS.

	CORPUS CALLOSUM.		CORTEX (PREFRONTAL).	
	Calculated in per cent.	Total found in per cent.	Calculated in per cent.	Total found in per cent.
Lecithin . . . . .	0.208	....	0.126	....
Kephalin and myelin .	0.129	....	0.027	....
Inorganic (det.) . . .	0.031	....	0.025	....
Sulphur compound . .	0.028	....	0.029	....
	0.396	0.418	0.207	0.19
Not accounted for + 0.022		....	-0.017	....
In per cent of total phosphorus + 5%		....	8%	....

NITROGEN.

	CORPUS CALLOSUM.		CORTEX (PREFRONTAL).	
	Calculated in per cent.	Total found in per cent.	Calculated in per cent.	Total found in per cent.
Lecithin . . . . .	0.093	....	0.057	....
Kephalin and myelin .	0.059	....	0.013	....
Cerebrin (1.9%) . . .	0.087	....	0.029	....
Sulphur compound . .	0.070	....	0.073	....
Extractives (det.) . . .	0.134	....	0.149	....
	0.443	0.439	0.321	0.333
Not accounted for 0.004		....	0.012	....
In per cent of total nitrogen -1%		....	+ 4%	....

For a better comparison of the relative composition of white and grey matter (with the exception of the inorganic salts), the following table, calculated in per cents of solids, is interesting :

	Corpus callosum.	Cortex (prefrontal).	Grey free from white.
Simple proteids . . . . .	10.0	27.71	21.7
Nucleoproteids . . . . .	11.56	16.66	9.66
Neurokeratin . . . . .	8.4	2.22	..
Extractives . . . . .	4.75	8.78	5.92
Lecithins . . . . .	16.22	17.44	7.67
Kephalin, myelin . . . . .	10.91	4.11	..
Phrenosin, kerasin . . . . .	14.29	8.61	..
Cholesterin . . . . .	15.2	3.89	..
Sulphur compound	4.37	8.06	5.43
Total solids . . . . .	32 per cent	18 per cent	

The third column of figures given above is obtained by calculating the amount of medullated nerve fibres (white) in the cortex, with phrenosin and kerasin as a basis and subtracting. Noll has shown that the substances (called by him protagon, now known to consist of cerebrins) which contain the reducing sugar are only present in the myelin sheath and not in the grey matter. An approximate idea of the quantity of white fibres present can therefore be arrived at from the amount of cerebrins (phrenosin and kerasin) in the cortex. Calculating now the amount of other constituents introduced with the fibres into the cortex, and subtracting them, the very interesting result is arrived at, that cortical grey matter, free from white fibres, besides containing no cerebrins, also contains no neurokeratin, cholesterin, and kephalin or myelin. Grey matter, therefore, has a very simple composition consisting of a mass of proteids, lecithin, and the sulphur compound. This result needs further confirmation by other analyses before any far-reaching conclusions can be based upon it. It serves, however, to indicate some of the possibilities of this method of attacking the problem.

In conclusion, it gives me great pleasure to express my thanks to Dr. F. W. Mott, F. R. S., for placing at my disposal the exceptionally fine resources of the Pathological Laboratory of the Claybury

Asylum, London, and to his assistant, Dr. Watson, for his many kind suggestions in the collecting of the material. The funds for carrying on the greater part of this work, and for collecting a large amount of valuable material, some of which still remains to be analyzed, were kindly supplied by the Rockefeller Institute for Medical Research.

# A PRELIMINARY STUDY OF THE DIGESTIBILITY OF CONNECTIVE TISSUE MUCOIDS IN PEPSIN- HYDROCHLORIC ACID.

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## HISTORICAL.

ABOUT nine years ago Gies made, at Chittenden's suggestion, an examination of the results of Loebisch's<sup>1</sup> study of tendomucoid. In the investigation published subsequently by Chittenden and Gies,<sup>2</sup> the proteid products resulting in acid hydrolysis of tendomucoid were found to be albumid, albuminate, proteoses, and peptone.<sup>3</sup>

Shortly after the publication of the paper giving these results, Gies tried the digestibility in pepsin-hydrochloric acid of some of his samples of pure tendomucoid. A few preliminary experiments made it evident that the substance was digestible in that medium. During the next few years numerous tests of additional preparations which had been made in connection with class instruction, gave the same results,<sup>4</sup> and favorable opportunity was awaited for detailed study of the zymolytic products.

At that period (1896-99), statements were current to the effect that connective tissue mucoids (*mucins*)<sup>5</sup> are "not digested by arti-

<sup>1</sup> LOEBISCH: Zeitschrift für physiologische Chemie, 1886, x, p. 40.

<sup>2</sup> CHITTENDEN and GIES: Journal of experimental medicine, 1896, i, p. 186.  
Also GIES and COLLABORATORS: Biochemical researches, 1903, i, Reprint No. 13.

<sup>3</sup> Similar products have been obtained by HAMMARSTEN and his pupils. See FOLIN: Zeitschrift für physiologische Chemie, 1897, xxiii, p. 345.

<sup>4</sup> These results were well known to the workers in this laboratory as early as 1895.

<sup>5</sup> No little confusion in our understanding of glucoproteid relationships has resulted in the past from indiscriminate use of the terms "mucin" and "mucoid." Thus, the glucoproteids in saliva and tendon have been regarded as *mucins*, and the similar product from cartilage is termed *mucoid*. Yet the resemblance between the glucoproteids obtainable from tendon and cartilage is much closer than that

ficial gastric juice," and that these substances are "insoluble in solutions of hydrochloric acid containing less than 1 per cent of the acid,"<sup>1</sup> although Schmiedeberg had shown that chondromucoid is converted, during artificial gastric digestion of cartilage, into a number of products, such as "peptochondrin" and "glutinchondrin,"<sup>2</sup> and Müller<sup>3</sup> had observed that the glucoproteid from the respiratory passages was also digested by pepsin-hydrochloric acid. We have since shown in this laboratory that the mucoids are soluble in hydrochloric acid of less strength than that of a 1 per cent solution.<sup>4</sup>

In the paper by Chittenden and Gies,<sup>5</sup> attention was called to the unexpectedly high sulphur content of the tendomucoid preparations described by them. Their results in this connection differed widely from those previously obtained by Loebisch.<sup>6</sup> Loebisch found 0.8 per cent of sulphur in his preparations; Chittenden and Gies, 2.3 per

between the glucoproteids in tendon and saliva. We have lately shown in this laboratory that the connective tissue glucoproteids are so much alike that at present they may be regarded as almost identical. Several years ago we wrote as follows regarding the glucoproteid from tendon: "Following COHNHEIM's suggestion (*Chemie der Eiweisskörper*, 1900, p. 259), we use the term 'mucoid,' *instead of the previously accepted 'mucin,'* to designate this substance. We agree with COHNHEIM that, for the sake of definiteness, the term 'mucin' may be best applied to the glucoproteids elaborated by true secretory cells, and the term 'mucoid' to similar substances in the tissues. In the present unsettled state of our chemical knowledge regarding these bodies, such a distinction is at best of only temporary convenience. The original differences have little importance in the light of the results of recent researches." See CUTTER and GIES: *This journal*, 1901, vi, p. 155; *Biochemical researches*, 1903, i, Reprint No. 5.

<sup>1</sup> HALLIBURTON: *Text book of chemical physiology and pathology*, 1891, pp. 479 and 481.

<sup>2</sup> SCHMIEDEBERG: *Archiv für experimentelle Pathologie und Pharmakologie*, 1891, xxviii, p. 355.

<sup>3</sup> FR. MÜLLER: *Jahresbericht über die Fortschritte der Thier-chemie*, 1896, xxvi, p. 6. MÜLLER's work gave results suggesting a close relationship between connective tissue mucoids and secreted mucins. His products from sputum yielded ethereal sulphuric acid, formic acid, and acetic acid, data similar to SCHMIEDEBERG's for chondromucoid. These substances were also obtained directly from submaxillary mucin. See also similar data for ovomucoid obtained by SEEMAN: *Jahresbericht über die Fortschritte der Thier-chemie*, 1898, xxviii, p. 18, and for mucins of the digestive tract by JACEWICZ: *Ibid.*, 1896, xxvi, p. 8. Compare, also, with the results subsequently obtained by PANZER and LEVENE, page 333.

<sup>4</sup> GIES and COLLABORATORS: *This journal*, 1901, v, p. 400; 1902, vii, p. 117; also, *Biochemical researches*, 1903, i, Reprints Nos. 3 and 4.

<sup>5</sup> CHITTENDEN and GIES: *Loc. cit.*

<sup>6</sup> LOEBISCH: *Loc. cit.*

cent in theirs. Unfortunately, Chittenden and Gies failed to appreciate the close relation of tendomucoid to chondromucoid suggested by their figures for its sulphur content and elementary composition in general.<sup>1</sup> The above-mentioned digestive results for tendomucoid, contrasted with those for chondromucoid published a few years previously by Schmiedeberg,<sup>2</sup> first suggested to Gies this relationship. The intimacy of this relation was then noted also in a comparison of the elementary composition of the two substances as expressed in the following figures for percentage content:

	C	H	N	S	O
Tendomucoid (Chittenden and Gies <sup>3</sup> ) . . . . .	48.76	6.53	11.75	2.33	30.63
Chondromucoid (Mörner <sup>4</sup> ) . . . . .	47.30	6.42	12.58	2.42	31.28

Occasional examinations of the osazone and sulphur-containing products obtainable from each also led, a few years ago, to the conclusions that chondromucoid and tendomucoid are more intimately related than had been supposed,<sup>5</sup> and that *all* the connective tissue mucoids are very much the same.<sup>6</sup>

This idea was strengthened by the publication of Hammarsten's<sup>7</sup> views on the "chondroproteids" (into which group chondromucoid and amyloid were placed because of the similarity of their proteid and ethereal sulphuric acid radicles), and also by Panzer's<sup>8</sup> observations, shortly afterward, on the properties of another "chondroproteid," viz., ovaricolloid, which likewise was found to contain an

<sup>1</sup> They said: "We present these figures with some doubt in our own minds, but, having obtained them as the result of most careful work, we see no possible explanation other than that this amount of sulphur is actually present in the molecule."

<sup>2</sup> SCHMIEDEBERG: *Loc. cit.*

<sup>3</sup> It has been shown frequently that LOEBISCH's figures for sulphur content of tendomucoid were too low. The higher figures obtained by CHITTENDEN and GIES have been verified repeatedly.

<sup>4</sup> C. TH. MÖRNER: *Skandinavisches Archiv für Physiologie*, 1889, i, p. 210.

<sup>5</sup> CUTTER and GIES: *Proceedings of the American Physiological Society*, 1899; this journal, 1900, iii, p. vi; 1901, vi, p. 155; also *Biochemical researches*, 1903, i, p. 38 and Reprint No. 5.

<sup>6</sup> CUTTER and GIES: *Loc. cit.*; also MEAD and GIES: *Proceedings of the American Physiological Society*, 1901; this journal, 1902, vi, p. xxviii; *Biochemical researches*, 1903, i, p. 53.

<sup>7</sup> HAMMARSTEN: *Lehrbuch der physiologischen Chemie*, 1899, p. 47.

<sup>8</sup> PANZER: *Zeitschrift für physiologische Chemie*, 1899, xxviii, p. 363 (see also the third footnote, page 331).

ethereal sulphuric acid radicle similar to chondroitin sulphuric acid.<sup>1</sup>

Some months after the publication of the very suggestive results obtained by Panzer, Levene<sup>2</sup> reported similar facts connected with tendomucoid, submaxillary mucin,<sup>3</sup> and carcinoma mucoid. The ethereal sulphuric acid product obtained by Levene was found to resemble chondroitin sulphuric acid in some respects, but also to differ from it in others, a discovery still further emphasizing the resemblance between chondromucoid and tendomucoid.

In the preparation of his glucothionic acid, Levene also found that tendomucoid was digestible in artificial gastric juice, although he did not give the results of any experiments to determine the chemical nature of the proteolytic products thus formed.

About the time Levene was obtaining his results, Leathes<sup>4</sup> identified a chondrosin-like substance among the hydrolytic products produced from paramucin. In its preparation from paramucin, the latter body was digested in pepsin-hydrochloric acid, but the proteolytic products were not described.

In Nerking's<sup>5</sup> work on "fat-proteid compounds," it was noted that ovomucoid and sub-maxillary and snail mucins are digestible in pepsin-hydrochloric acid. The products of digestion were not studied.

Shortly after Gies's discovery of osseomucoid,<sup>6</sup> it was shown in this

<sup>1</sup> Ethereal sulphate was also obtained from tendomucoid by CHITTENDEN and GIES, but its significance was not comprehended until the comparisons were made in the course of the further study alluded to above. Their results in this connection were referred to briefly as showing that the greater portion of the sulphur was "closely combined (*i. e.*, as  $\text{SO}_4$ ), a small amount only being in the form of the mercaptan group."

<sup>2</sup> LEVENE: *Journal of the American Chemical Society* (Preliminary communication), 1900, xxii, p. 80; *Zeitschrift für physiologische Chemie*, 1901, xxxi, p. 395; 1903, xxxix, p. 1. Also, *Studies from the Department of Physiological Chemistry in the Pathological Institute of the New York State Hospitals*, 1902-1903, Reprint No. 8 (see also the third footnote, page 331).

<sup>3</sup> LEVENE's results with submaxillary mucin extend the earlier data of a similar nature published by MÜLLER: *Jahresbericht über die Fortschritte der Tierchemie*, 1896, xxvi, p. 6.

<sup>4</sup> LEATHES: *Archiv für experimentelle Pathologie und Pharmakologie*, 1899, xliii, p. 245.

<sup>5</sup> NERKING: *Archiv für die gesammte Physiologie*, 1901, lxxxv, p. 330.

<sup>6</sup> GIES: *Proceedings of the American Physiological Society*, 1899; this journal, 1900, iii, p. vii; also *Biochemical researches*,<sup>8</sup> 1903, i, p. 31.

laboratory that the glucoproteid from bone is also digestible in pepsin-hydrochloric acid.<sup>1</sup> Similar facts were ascertained recently with regard to the mucoids from ligament<sup>2</sup> and cartilage.<sup>3</sup>

Four years ago we together took up the question of mucoid digestibility. The results presented below have been obtained in the preliminary experiments carried out occasionally in the mean time.<sup>4</sup>

In most of our experiments tendomucoid was employed. Some facts on the digestibility of the mucoids from bone, cartilage, and ligament are also given.

#### PREPARATION OF THE MUCOIDS.

**Tendomucoid.**—This was prepared by the customary method of extraction from washed tendon sections in half-saturated lime-water, and precipitation of the filtered extract with 0.2 per cent hydrochloric acid. The sheath and shaft of the Achilles tendon of the ox were employed.<sup>5</sup> The precipitated mucoid was thoroughly washed with water until free from acid, dehydrated in alcohol, and then usually dried to thin scales on dishes at room temperature.<sup>6</sup> Large quantities of tendons were used in each preparation, and several hundred grams of mucoid prepared at one time. The dried mucoid was much like dry gelatine in appearance,—hard, pliable, dark in color, and quite transparent. In this condition it readily absorbed water, and when immersed in it, the mucoid very soon became opaque, swollen, white, soft, and, on stirring, flocculent, like the freshly precipitated substance. It behaved in the same way when suspended in 0.2 per cent hydrochloric acid. In some of our experiments the freshly precipitated material was used directly.

While this work was in progress, Cutter and Gies<sup>7</sup> obtained results

<sup>1</sup> HAWK and GIES: This journal, 1901, v, p. 401; Biochemical researches, 1903, i, Reprint No. 3. See also KRAWKOW: Archiv für experimentelle Pathologie und Pharmakologie, 1897, xl, p. 195.

<sup>2</sup> RICHARDS and GIES: This journal, 1902, vii, p. 116; Biochemical researches, 1903, i, Reprint No. 4.

<sup>3</sup> POSNER and GIES: This journal, 1902, vii, p. 331; Biochemical researches, 1903, i, Reprint No. 35.

<sup>4</sup> Some experiments in this connection were also begun by MEAD and GIES, but these were unavoidably interrupted. See Proceedings of the American Physiological Society, 1901; this journal, 1902, vi, p. xxviii; also Biochemical researches, 1903, i, p. 53.

<sup>5</sup> CUTTER and GIES: *Loc. cit.*

<sup>6</sup> This occurred in several hours.

<sup>7</sup> CUTTER and GIES. *Loc. cit.*



which led them to conclude that tendon contains more than one glucoproteid. They observed not only that the mucoids from the various parts of the Achilles tendon were different in composition, but also that successive lime-water extracts from the same parts of the tissue yielded mucoids of different composition and requiring increased amounts of acid for their precipitation. We have verified these results.

Below we give the figures for content of ash and nitrogen (ash-free substance) of three mucoid fractions prepared from the same supply of tendon pieces (76 lbs.). Each product required an increased quantity of acid for its precipitation. The results are also compared with sample data from those given by Cutter and Gies.

COMPOSITION OF MUCOIDS OBTAINED BY THE FRACTIONAL METHOD.

MUCOID.	NITROGEN. <sup>1</sup>			ASH.		
Prepared by	Fractions					
	1	2	3	1	2	3
Cutter and Gies <sup>2</sup>	12.64	12.69	13.91	0.75	1.78	1.28
Posner and Gies <sup>3</sup>	12.03	12.34	12.97	0.37	0.34	1.02

<sup>1</sup> In the experiments of CUTTER and GIES periods of extraction different from those referred to below gave mucoid having the lowest nitrogen content in the *second* fraction.

<sup>2</sup> "Series D" (p. 160) obtained from the "sheath." Periods of extraction in half-saturated lime-water, 2 c.c. per gram of tissue: First, 17 hrs.; second, 20 hrs.; third, 26 hrs.

<sup>3</sup> Obtained from whole tendons ("shaft" and "sheath"). Periods of extraction in half-saturated lime-water, 4 c.c. per gram of tissue: First, 48 hrs.; second, 96 hrs.; third, 168 hrs.

**Other mucoids.** — Preparations of glucoproteid from cartilage, ligament and bone, which had been prepared for previous investigations in this laboratory, were at our disposal for several experiments. These products were separated and purified by methods already described.<sup>1</sup>

<sup>1</sup> HAWK and GIES: *Loc. cit.* (osseomucoid); RICHARDS and GIES: *Loc. cit.* (ligamentomucoid); POSNER and GIES: *Loc. cit.* (chondromucoid).

## DIGESTION EXPERIMENTS.

The essential results of our peptic digestions are briefly summarized below.

**First experiment.** — Two samples of dried tendomucoid were taken. Each weighed 15 grams. Digestion proceeded at 40° C. in 1800 c.c. of 0.2 per cent hydrochloric acid containing 0.2 per cent commercial pepsin.<sup>1</sup>

*Sample A.* — After four days, much of the mucoid had disappeared into a yellowish solution, leaving behind a brown *flocculent* residue. Free acid was present in the solution. On neutralizing the filtrate the fluid remained perfectly clear — acidalbumin absent. On saturating the filtrate with ammonium sulphate, a heavy precipitate of proteoses was obtained. The filtrate from the proteoses was evaporated to a thin syrup, and peptone separated and identified with Kühne's method.<sup>2</sup> The amount of peptone was quite small.

Proto-, hetero-, and deuteroproteoses were separated from the crude proteose mass, and identified with Kühne's methods.<sup>3</sup> Secondary proteose predominated.

*Sample B* was digested for seven days. Free acid was present continuously. The results were qualitatively the same as before. The residue was less in amount, the primary proteose was diminished also. The secondary proteoses and peptone were increased.

*Indigestible matter.* — The residues from both samples were combined and treated with 0.25 per cent sodium carbonate in which the substance readily dissolved. On neutralization and further acidification with 0.2 per cent hydrochloric acid a heavy white flocculent precipitate was thrown down. After precipitation in this way, this albumid-like body in the moist condition had the general appearance of mucoid itself.

**Second experiment.** — Three samples of freshly precipitated tendomucoid were taken. The substance was prepared from a second extract<sup>4</sup> of ten-

<sup>1</sup> All of the digestions were carried out in flasks covered with watch-glasses and placed in an incubator. The flasks were repeatedly uncovered and shaken. The pepsin used in all of these experiments was taken from samples of the "1-2000" product prepared by Parke, Davis, and Co. Each pepsin-hydrochloric acid solution prepared by us manifested strong proteolytic action. The pepsin preparations were free from sulphate and did not yield reducing substance on hydration with acid.

<sup>2</sup> See HOPPE-SEYLER (THIERFELDER): *Handbuch der physiologisch- und pathologisch-chemischen Analyse*, 1903, p. 326.

<sup>3</sup> *Ibid.*, p. 321.

<sup>4</sup> CUTTER and GIES: *Loc. cit.*

don. Each sample contained about 35 grams of the moist precipitate. Digestion was carried out at 40° C., in 700 c.c. of an acid-pepsin solution equal in strength to that used in the previous experiment, and continued three days. Free acid was present to the end.

Acidalbumin was absent. The proteose and peptone results were identical with those of the first experiment, except that a larger proportion of primary proteose was found in this experiment. Kühne's methods<sup>1</sup> were used for separating these products.

The residue from each sample in this experiment was brown and soft, having a *doughy consistency*. It was sticky and gummy while moist at 40° C., but on drying the moist precipitate, at room temperature, it was hard and quite black (mucoid dried under similar circumstances is light brown in color). It showed the same qualities as those noted for the residues of the first experiment.

**Third experiment.** — Three samples of dried tendomucoid, each weighing exactly 20 grams, were digested three days at 40° C. in 1000 c.c. of 0.2 per cent hydrochloric acid containing 0.1 per cent commercial pepsin. Free acid was present throughout.

The usual qualitative results were obtained. In this experiment the residue was the same in appearance as that of the first. A small amount of albuminate was separated, and some dysproteose was detected with the other proteoses and peptone. Kühne's methods<sup>1</sup> of separation were again employed.

The indigestible substance in the three flasks was thoroughly washed with water<sup>2</sup> and alcohol, and dried. The combined products weighed 12 grams. Thus a total of 48 grams of the original substance had "digested," under the conditions of the experiment, a quantity equal to 80 per cent of the tendomucoid.

Eleven grams of the residue were again subjected to digestive influences. The material was repeatedly shaken in 800 c.c. of 0.2 per cent hydrochloric acid containing 0.25 per cent commercial pepsin, and the digestive process continued at 40° C. for five days.

At the end of that time free acid was present, and considerable residue remained. This was no longer flocculent, but doughy, as in the second experiment. All traces of unaltered mucoid had disappeared.

The residue was again treated with a fresh portion of the previous digestive mixture. Digestion continued two days under the usual condi-

<sup>1</sup> See HOPPE-SEYLER (THIERFELDER): *Handbuch der physiologisch- und pathologisch-chemischen Analyse*, 1903, p. 326.

<sup>2</sup> During this process it was observed that the interior of some of the larger flakes was white, a fact suggesting the presence of unaltered mucoid.

tions. The appearance of the residue remained about the same. When dry, it was black. It weighed 3.8 grams.<sup>1</sup>

By the end of the third digestion 55.2 grams<sup>2</sup> of the original substance had gone into solution in the pepsin-hydrochloric acid — at least 92 per cent was digested.

All of these residues resembled albumid. Analytic results are tabulated on page 347.

**Fourth experiment.** — Three portions of dry tendomucoid, each weighing exactly 25 grams, were digested for a week at 40° C. in 800 c.c. of 0.2 per cent hydrochloric acid containing 0.06 per cent commercial pepsin. Free acid was present throughout.

Although the volume of digestive fluid was less than before, and the percentage amount of pepsin much reduced, no albuminate was detectable in the filtrate from the residue. The latter had the appearance of that of the first experiment. The quantity of primary proteoses was less than we expected. The amounts of secondary proteose and peptone were greater than usual. Kühne's methods<sup>3</sup> were used for the separations.

The combined dry residues weighed 18.7 grams. A total of 56.3 grams of the mucoid had disappeared into solution — a digestion of 75.1 per cent. Seventeen grams of the residue were again subjected to digestion, this time for eleven days at 40° C. in 1000 c.c. of 0.2 per cent hydrochloric acid containing 0.2 per cent commercial pepsin. At the end of that long period, with free acid present in abundance and the pepsin very active, a residue of 7.5 grams still remained. By this time 65.8 grams of the original substance had disappeared into soluble products, — a digestion of at least 87.7 per cent.

The properties of the final residue were the same, at this stage, as in the previous experiment. See analytic data, page 347.

**Fifth experiment.** — In this experiment we used the mixed tendomucoids prepared fractionally with the method of Cutter and Gies, and referred to on page 335. The quantity of mucoid was the largest taken thus far. It was divided into five equal portions, and each was digested two days at 40° C. in 800 c.c. of 0.2 per cent hydrochloric acid containing 0.05 per cent commercial pepsin. Free acid was never absent.

The usual qualitative results were obtained. Considerable residue remained. The filtrate was free from albuminate, but contained a large

<sup>1</sup> All of the filtrates in these and subsequent experiments showed very strong digestive power when tested with fibrin. This was not ascertained in the previous experiments.

<sup>2</sup> One gram was used for analytic purposes.

<sup>3</sup> HOPPE-SEYLER (THIERFELDER): *Loc. cit.*

quantity of proteose, chiefly secondary, only a moderate amount of proto-proteose, less heteroproteose, hardly any peptone. Kühne's methods<sup>1</sup> were used for identifying these bodies.

In the moist condition the residue was flocculent, brown in color, with snow white interior (undigested mucoid). After drying, it was brownish black. The combined dry residues weighed 46.5 grams.

Forty-five grams of this residue were again subjected to digestive influences. This quantity was divided into two equal parts and each treated with 1000 c.c. of 0.2 per cent hydrochloric acid containing 0.1 per cent commercial pepsin. Digestion continued for six days at body-temperature. Free acid was present continuously. By the end of that period the appearance of the residue had altered very little, although the total quantity had decreased to 15 grams. The final residue was flocculent in the moist condition.

Analytic data are tabulated on page 347.

**Sixth experiment.** — A relatively large quantity of freshly prepared, moist tendomucoid, corresponding to about 100 grams of dry material, was divided into four equal parts, and each portion digested two days at 40° C. in 800 c.c. of 0.2 per cent hydrochloric acid containing 0.6 per cent commercial pepsin.

Considerable digestion occurred. The residue was white and granular. After drying, it weighed 29 grams. Acidalbumin could not be detected in the filtrate. Proteoses and peptone were separated with Kühne's method.<sup>2</sup> The primary proteoses were separated with Pick's method.<sup>3</sup> In which process considerable heteroproteose was obtained, though proto-proteose predominated.

Twenty-eight grams of the residue were digested five days more, under the conditions previously prevailing. At the end of that time 13 grams still remained undissolved. The second residue was still granular and a dark gray in color.

Twelve grams of the second residue were again subjected to the same digestive conditions for another period of five days. A residue of 5 grams persisted.<sup>4</sup> In the moist condition this was finely granular; when dry it was dark brown.

Free acid was continuously present in all of the digestive fluids of this experiment. Analytic data are given on page 347.

<sup>1</sup> HOPPE-SEYLER (THIERFELDER): *Loc. cit.*

<sup>2</sup> *Ibid.*

<sup>3</sup> *Ibid.*, p. 323.

<sup>4</sup> The alcoholic washings of the residues deposited, on standing, a light, white gelatinous precipitate. The amount was small. Its nature was not determined.

**Seventh experiment.** — From 4 to 10 grams of dry samples of osseomucoid, chondromucoid, and ligamentomucoid were subjected to digestive conditions equivalent to those of the first experiment.<sup>1</sup> Free acid was present throughout each digestion.

Albuminate could not be detected in the filtrates from the residues. Proteoses and peptone were identified with Kühne's methods. Secondary proteose predominated.

The residues were brown and doughy, while moist; dark brown and hard, when dry.

**Eighth experiment.** — Portions of the proto-, hetero-, and deuteroproteoses and the peptones of the fourth and sixth experiments were combined and separated with Pick's method.<sup>2</sup> Proto- and heteroproteoses, deuteroproteoses A, B, and C, and peptones A and B were obtained in the process. Deuteroproteose B greatly predominated over deuteroproteose A and C, and all other products.

**Ninth experiment.** — A mixture of samples of the various residues available, amounting to 9 grams, was subjected to digestion for four days at 40° C., in 500 c.c. of 0.2 per cent hydrochloric acid containing 0.2 per cent commercial pepsin. Most of the residue remained insoluble and was unchanged in appearance. The filtrate was free from albuminate, but contained secondary proteoses and peptones, though in relatively small amount.<sup>3</sup> The residue retained its usual properties, including that of yielding reducing substance in abundance on hydration with dilute acid (see page 342).

**General results and conclusions.** — The preceding experiments gave digestive results in perfect harmony with the qualitative data previously obtained by Gies.<sup>4</sup> In all cases, as soon as the digestive mixture became lukewarm, the mucoid began to diminish, the density of the solution immediately above the mucoid was seen, on shaking, to be greater than that of the rest of the fluid, the snow-whiteness of the solid gradually disappeared, the residue changed its consistency and increased its brownish color, and the solution deepened in a yellowish brown tinge, such as fairly concentrated solutions of proteoses always exhibit.

The digestive media were so active, and the periods of digestion

<sup>1</sup> POSNER and GIES: *Loc. cit.*

<sup>2</sup> HOPPE-SEYLER (THIERFELDER): *Loc. cit.*, p. 322.

<sup>3</sup> This quantity was greater than any that may have been introduced with the pepsin.

<sup>4</sup> GIES: See page 330.

relatively so long, that acidalbumin was almost always absent, although, as indicated in the third experiment, it is undoubtedly formed as an intermediate product. Albumid-like products, primary and secondary proteoses, and peptone invariably resulted. As a rule, the quantity of secondary proteoses obtained was greater than that of any of the other soluble products.

The foregoing results make it evident that the connective tissue mucoids are digestible in gastric juice. That mucoids are more resistant to the action of pepsin-hydrochloric acid than many other proteids is very evident, however, from these experiments, for the proportion of insoluble matter was always quite large, even after long intervals of treatment with particularly vigorous artificial gastric juice.<sup>1</sup> At best, digestion is relatively slow and gradual under the conditions of these experiments.<sup>2</sup>

#### ON THE NATURE OF THE INDIGESTIBLE MATTER.

In all of our pepsin-hydrochloric acid digestions of the connective tissue mucoid under examination, a fairly large proportion of the material remained insoluble. We have already alluded to the fact that this residue possessed albumid-like properties, but it was unlike albumid in yielding much reducing substance, and considerable

<sup>1</sup> We have had several mucoid preparations digesting at room temperature, in the original digestive mixture, for about two years. At the end of that time free acid was present, the fluid had a strong solvent action on fibrin, but the residues were apparently almost as large in amount as they had been after the first week's digestion.

<sup>2</sup> Trypsin appears to digest mucoid much more rapidly and thoroughly than pepsin.

(a) In one of our experiments in this connection 10 grams of crude tendomucoid were digested two days at 40° C. in 250 c.c. alkaline tryptic solution (0.25 per cent Na<sub>2</sub>CO<sub>3</sub>). At the end of that time practically all the substance had gone into solution, albuminate was missing, only a trace of proteose could be separated, but a fairly large proportion of peptone could be detected. A very strong tryptophan reaction was given by the fluid, and tyrosin was conspicuous among the crystalline products. The peptone separated with KÜHNE'S method failed to yield reducing substance on hydration with dilute acid (see page 348).

(b) Three grams of residue obtained in previous peptic digestions were subjected to the treatment detailed under (a), with practically the same results. All of the material was converted into peptone and crystalline products. The tryptophan reaction was only very faint, only a trace of proteose was detectable, and tyrosin was abundant. The peptone separated with KÜHNE'S method again failed to yield reducing substance after acid decomposition (see page 348).

etheral sulphate *after decomposition with 2 per cent hydrochloric acid*. The doughy consistency and other properties of these residues at times reminded us of Schmiedeberg's peptochondrin<sup>1</sup> and led us to look, with success, for the above radicles on decomposition.

It is now well known that the chondroitin sulphuric acid obtainable from chondromucoid, and the glucothionic acids from various other mucoids, share the property of combining with proteoses and other proteids to form soluble or insoluble products in the presence of free acid, or under other conditions. That our indigestible residues were such compounds is certain.<sup>2</sup>

Analytic data are tabulated on page 347.

In the belief that the various digestive residues were glucothionic acid compounds similar to Schmiedeberg's peptochondrin rather than typical albumid, we made several attempts to isolate such substances from the insoluble products. Our results are briefly summarized below.

**A. Schmiedeberg's method** of isolating chondroitin sulphuric acid was first tried. Portions of the digestive residues obtained in the first and second experiments were treated with 2.5 per cent hydrochloric acid at room temperature for about twenty-four hours. A dark brown turbid solution resulted. A very small amount of unidentified matter remained insoluble even after long standing and repeated stirring.<sup>3</sup> On treating the filtrate with one-fourth its volume of 95 per cent alcohol, it remained perfectly clear. The substance in this filtrate had no reducing action. After boiling with 2 per cent hydrochloric acid, however, an abundance of reducing substance was produced. The above alcoholic filtrate was next treated, in excess, with a mixture of four volumes of absolute alcohol and one of ether, when a heavy white flocculent precipitate was obtained.<sup>4</sup> This material dissolved readily in dilute potassium hydroxide to a brown solution. On adding to the latter four volumes of 95 per cent alcohol, a voluminous white gelatinous precipitate was thrown down. The filtrate was yellowish in color.<sup>5</sup>

<sup>1</sup> SCHMIEDEBERG: *Loc. cit.*

<sup>2</sup> See page 349, for facts relating to our proteoses in this connection; also facts regarding "chondralbumin": SCHMIEDEBERG, *Loc. cit.*

<sup>3</sup> On *warming* this substance in 2.5 per cent hydrochloric acid, practically all of it dissolved. On decomposing it thoroughly in this acid, no reducing substance was obtained from it.

<sup>4</sup> On adding to the filtrate a small quantity of 10 per cent solution of sodium chloride, an additional precipitate, presumably of the same substance, was formed. This product was not admixed with the first precipitate, however.

<sup>5</sup> On neutralizing this filtrate, no precipitation occurred. On rendering it acid, the yellow color disappeared and a slight turbidity ensued.



a. The precipitate obtained in this way was found to be readily soluble in cold water. The solution was brownish and turbid. The turbidity did not increase perceptibly on boiling. The watery solution of the precipitate had no reducing action. After boiling a few minutes with 2 per cent hydrochloric acid, a strong reducing power was manifested, and an abundance of ethereal sulphate was detected. The precipitate seemed to be a crude basic potassium-glucothionate.

At this point our product was divided into two approximately equal parts. One-half was used for the preparation of a potassium-copper salt. The other was treated with Levene's method, as indicated under B.

b. *Potassium-copper compound.*—The portion of the precipitate reserved for this preparation was first dissolved in water. This solution was treated successively with a moderate excess of copper acetate, then with potassium hydroxide until a deep violet color was produced, and next with a large excess of 97 per cent alcohol, when a heavy, bluish green, flocculent precipitate was thrown down. This precipitate was dissolved in water, and the solution again poured into an abundance of 97 per cent alcohol; but only turbidity resulted, until the mixture was made faintly alkaline with potassium hydroxide, when the potassium-copper precipitate was again obtained in large flakes. The substance was re-precipitated a second time, thus freeing it almost completely from proteid radicles.

This compound was readily soluble in water, and gave a slight biuret reaction. Added to an aqueous solution of Witte's peptone, no precipitate resulted. On acidifying the mixture with acetic acid, however, a heavy precipitate formed at once. The acetic acid had no such effect on either the aqueous solutions of our compound or of the Witte's peptone. When the acidified solutions were mixed, heavy precipitates resulted. After decomposition in 2 per cent hydrochloric acid, heavy reduction of Fehling's solution was manifested, and an abundance of sulphate precipitated as barium sulphate.

The average amount of nitrogen contained in this product was 4.84 per cent. These figures are high, doubtless because of the presence of admixed proteid, yet their nearness to those for the same compound of chondroitin-sulphuric acid is significant.

B. **Levene's method** was next employed. One-half of the precipitate obtained, as indicated under "a" above, was dissolved in 2 per cent potassium hydroxide solution and allowed to stand nearly forty-eight hours. At the end of that interval, the alkali was nearly neutralized with acetic acid, the faintly alkaline solution was treated with a moderate excess of picric acid, and then made strongly acid with acetic acid. The filtrate from a slight precipitate was directed into 4-5 volumes of 95 per cent alcohol, and a heavy white gelatinous precipitate obtained. Nearly all of

the precipitate dissolved readily in water, and the process was repeated with the filtrate.

The aqueous solution of the substance thus separated was acid to litmus and gave a slight biuret reaction. The substance yielded reducing material and ethereal sulphate on decomposition, and showed the same behavior in the presence of Witte's peptone and acid as that indicated for the potassium-copper compound under "b" on page 343.

The nitrogen content was found to be 5.22 per cent.

C. **Levene's method combined with Mörner's method** was also used on two different mixtures of digestive residues designated below, I and II.

I a. *Levene's method.* — The remaining portions of the second residues obtained in the fourth and fifth experiment were combined and dissolved in 10 per cent sodium hydroxide. A greenish brown, slightly turbid fluid resulted. The process indicated in the first paragraph under "B" was duplicated at this point, except that sodium hydroxide was used instead of the caustic potash, and the acid substance was reprecipitated a second time.

I b. *Mörner's method.* — The precipitate thus obtained and corresponding in the main to that produced under "B" was next entirely dissolved in 5 per cent sodium hydroxide, to a yellowish brown solution. This was neutralized with acetic acid, the fluid remaining entirely clear. Tannic acid was then added in moderate excess, when only a slight turbidity resulted. On adding acetic acid in small quantity, the turbidity was not appreciably increased. Tannic acid was removed from the filtrate with lead acetate; the excess of lead was precipitated from the next filtrate with hydrogen sulphide gas. The lead-free filtrate was neutralized, concentrated on the water bath at a low temperature, and the thin syrup that resulted was poured into a large volume of alcohol. Only heavy turbidity resulted at first. In a short time, however, a flocculent precipitate settled out.

I c. *Levene's method.* — The precipitate obtained by the above purification process was dissolved in water and Levene's method again applied to it.

The precipitate that resulted was dissolved in water to a clear solution. This solution, whether neutral or acid, gave no further precipitation with either picric acid or tannic acid. The solution was finally treated with alcohol in excess, and the precipitate washed and dehydrated for the usual analysis. 2.2 grams of purified substance were obtained.

I d. The aqueous solution of the substance was acid in reaction. With potassium hydroxide and copper acetate it gave the product obtained under "A, b." The product appeared to be *entirely free from biuret reacting material*. A proteose solution was not precipitated by a neutral

watery solution of our compound; an *acidified* solution of proteose was heavily precipitated by it. The usual reduction and sulphate reactions were obtained after decomposition in 2 per cent hydrochloric acid.

Our analytic results were the following:

Ash	= 2.05 per cent
Nitrogen	= 2.95 " "
Sulphur	= 5.91 " "

II a. *Mörner's method.* — Combined residues obtained in the third experiment, amounting to 15.5 grams, were dissolved in 300 c.c. of 3.5 per cent sodium hydroxide. An abundant precipitate of glucothionic acid was obtained by Mörner's method, as above.

II b. *Levene's method.* — The alkaline solution of the substance prepared by the preceding process was treated with sodium hydroxide, and Levene's method carried forward. The final substance was reprecipitated twice. 3.4 grams were obtained, — a quantity equal to 23.9 per cent of the residue taken at the beginning.

This product seemed to be identical with the one referred to under "I d."

The following analytic results were obtained:

Ash	= 2.79 per cent
Nitrogen	= 2.43 " "
Sulphur	= 6.66 " "

**General results and conclusions.** — The residues obtained in the seventh experiment from chondromucoid and osseomucoid contained an abundance of the carbohydrate radicles, gave strong reactions for sulphate after decomposition with hydrochloric acid, and appeared to be practically identical qualitatively with the residue from the tendomucoid. The residue from the ligamentomucoid was not examined in this particular connection, but its appearance indicated that identical results would have been obtained with it.

In our last two separations, under "C," the products were practically free from proteid radicles, as was indicated by negative biuret reactions, and they appeared to be acid substances directly comparable to chondroitin sulphuric acid. On the next page we give comparative figures:

## NITROGEN AND SULPHUR IN GLUCOTHIONIC ACIDS.

Substance. <sup>1</sup>	Formula.	Analyzed by	Nitrogen.	Sulphur.
1. Chondroitin sulphuric acid . . . .	$C_{18}H_{27}NSO_{17}$	(Calculated)	2.49	5.71
2. Glucothionic acid from mucoid digestive residues . . . .	. . . .	Posner and Gies	$\left\{ \begin{array}{l} I \ 2.95 \\ II \ 2.43 \end{array} \right.$	$\left\{ \begin{array}{l} 5.91 \\ 6.66 \end{array} \right.$
3. Chondroitin copper sulphate . . . .	$C_{18}H_{25}CuNSO_{17} + H_2O$	(Calculated)	2.18	5.00
4. Chondroitin copper sulphate . . . .	$C_{18}H_{25}CuNSO_{17} + H_2O$	Schmiedeberg	2.45	4.96

<sup>1</sup> Similar results are given by MÖRNER: *Skandinavisches Archiv für Physiologie*, 1889, i, p. 210; also, LEVENE: *Zeitschrift für physiologische Chemie*, 1901, xxxi, p. 402.

A comparison of the above results with those obtained by Schmiedeberg, Levene, and others, makes it evident, we think, that our residues contained a radicle similar to chondroitin sulphuric acid. Our analytic data show hardly any more variation than those obtained by previous investigators, and it is quite as likely that variable double compounds or mixtures were produced and analyzed in these experiments, as in the work of Schmiedeberg and of Levene. Schmiedeberg found that the glucothionic acid from cartilage decomposes readily, forming mixtures, *e.g.*, of chondroitin and chondroitin sulphuric acid; that it is very difficult to remove proteid from it, that it unites easily with other impurities, and that its compounds are transformed chemically to a certain extent even on drying in a desiccator over sulphuric acid at ordinary temperatures.<sup>1</sup>

The residues left behind in our digestive experiments are evidently compounds containing proteid and glucothionic acid radicles. Whether the residues represent resistant fragments of the mucoid molecule, or whether they are formed anew by a different union of proteid and glucothionic acid radicles split off in the digestive process, can only

<sup>1</sup> Differences between the glucothionic acids from chondromucoid and tendomucoid have been brought out, lately, in the experiments of ORGLER and NEUBERG (*Zeitschrift für physiologische Chemie*, 1903, xxxvii, p. 407) and of LEVENE (*Ibid.*, 1903, xxxix, p. 1; also *Studies from the Department of Physiological Chemistry of the Pathological Institute of the New York State Hospitals*, 1902-1903, Reprint No. 8).

be guessed at present. That they may result from such interactions of digestive fragments is evident from the fact that the glucothionic acids of the type here considered form various kinds of pseudo-mucoid products, such as Schmiedeberg's peptochondrin, when brought into contact with gelatine, proteoses, etc., in acid solutions.

Thus far we have not attempted to ascertain the nature of the proteid radicles associated in the residues with the glucothionic acid.

Further facts regarding the digestive residues may be noted from the figures tabulated below. The nitrogen content of each residue was always less than that of the mucoid itself, and after each digestion the subsequent residue had a still smaller nitrogen content.

These facts are in harmony with our preceding deductions, and indicate strongly that the proteid radicles were relatively decreased, either in number or complexity, or both, as the peptic treatment of the residue continued.

PERCENTAGE CONTENT OF NITROGEN IN TENDOMUCOIDS AND DIGESTIVE PRODUCTS.<sup>1</sup>

EXPERIMENT. No.	ORIGINAL TENDO- MUCOID.	UNDIGESTED RESIDUE.		PRIMARY MU- COPROTEOSE. <sup>2</sup>		SECOND- ARY MU- COPRO- TEOSE. <sup>2</sup>	MU- COPEPTONE. <sup>2</sup>
		First.	Second.	Proto.	Hetero.		
III	12.31	10.45	9.91	15.27		10.70	—
IV	12.31	10.73	9.70	—	—	12.70	13.89
V	12.18	10.74	10.08	13.43	12.91	9.85	—
VI	12.82	11.71	11.04	12.46		—	13.81

<sup>1</sup> Ash-free substance. The analyzed substance had been dried to constant weight at 110° C. The figures are averages of closely agreeing duplicates.

<sup>2</sup> See page 348.

As a rule the percentage of nitrogen in the peptones is less than in the original proteids from which they were produced.<sup>1</sup> The quantity of nitrogen in the mucopeptones is relatively higher than in the mucoids. This difference is due to the fact that the mucoid contains the glucothionic acid radicle (with its small nitrogen content), whereas the mucopeptone does not. It is very probable that

<sup>1</sup> CHITTENDEN: Digestive proteolysis, 1895, p. 67 *et seq.*

the nitrogen content of the proteid part of the mucoid molecule is considerably higher than that of our mucopeptones.

#### ON THE PROPERTIES OF THE SOLUBLE DIGESTIVE PRODUCTS.

So little albuminate was obtained in these experiments that no special attention was paid to it.

Our various mucoproteoses<sup>1</sup> gave the well-known general reactions characteristic of the group to which each belonged. Thus the primary mucoproteoses, as separated with Kühne's method, were precipitated by concentrated nitric acid, and by acetic acid and potassium ferrocyanide; the secondary products were not. The latter were readily precipitated by trichloroacetic acid and by potassium mercuric iodide. All of these precipitates disappeared on warming and reappeared on cooling. Heteromucoproteose was separable on dialysis of its saline solutions, and was insoluble in water. On one occasion dysproteose was formed (page 337).

The secondary mucoproteoses always predominated in amount in these experiments. The quantity of peptone was usually small. The physical properties of the various products were about the same as those of typical proteoses from other sources.

On decomposing with acid, and testing the products with Fehling's solution in the usual way, variable reduction results were obtained.

The mucopeptones always failed to yield reducing products. The neutralized digestive filtrate was without reducing action, but after acid decomposition it gave a strong reduction of Fehling's solution.

In one instance protomucoproteose and in another heteromuco-

<sup>1</sup> HAMMARSTEN has prepared proteoses and peptones from various glucoproteids by treating them with alkali and acid. He designates the former *mucinalbumoses*, the latter, *mucinpeptone*. He has also separated from ascites fluid a product termed by him *mucinalbumose* (*Zeitschrift für physiologische Chemie*, 1891, xv, p. 206; see also FOLIN: *Ibid.*, 1897, xxiii, p. 360). It appears to us that the terms *mucoproteose* and *mucopeptone* are, perhaps, better adapted for designating the proteoses and peptones obtained from the *mucoids*. We do not know that the proteid part of the glucoproteid molecule consists of *albumin*. The term "glucoproteose" would also be unsatisfactory in this connection, because it has already been applied to deuteroproteose B prepared from fibrin and other proteids with the Pick method (*Beiträge zur chemischen Physiologie und Pathologie*, 1902, ii, p. 481). "Mucose," already used in another connection by MÜLLER (*Loc. cit.*), would be apt to suggest a carbohydrate rather than a proteid product. The current use of such names as *albumoses*, *caseoses*, *myosinoses*, *globuloses*, etc., warrants also, we think, the employment of the descriptive terms *mucoproteoses* and *mucopeptones*, as proposed above.

proteose slightly reduced Fehling's solution *before* their decomposition with dilute acid. In other instances, reducing power was manifested only *after* decomposition with acid. Occasionally *no reduction* of Fehling's solution could be observed even after decomposition of the proteose products in the usual manner. The deuteromucoproteose obtained with Kühne's method yielded abundant reducing substance *after* hydration, not before.<sup>1</sup> Crystalline osazone products were obtained in several instances when the products reduced Fehling's solution. These crystals resembled those of glucosazone. Deuteromucoproteoses B and C, obtained with Pick's method, reduced Fehling's solution after their decomposition with acid. The reduction was especially evident after removal of the proteid products from the reducing material, with Mörner's method of separating chondroitin sulphuric acid.

At present we are unable to state the reasons for the observed variability in reducing powers of our proteose products after decomposition, but different conditions of digestion will doubtless be found to have an important bearing in this connection.

In testing for loosely united sulphur, it was found that the primary mucoproteoses and deuteromucoproteose B gave very strong reactions. Deuteromucoproteose C did not give the reaction at all. Product A was not available. Mucopeptones A and B also failed to respond to the reaction with potassium hydroxide and lead acetate.

The above reduction results suggested that the various mucoproteoses in some cases contained, or were combined with, the glucothionic acid radicle separated previously from the insoluble matter.

In one experiment, to test this probability, portions of primary mucoproteoses, obtained in the fifth and sixth experiments, were suspended in water, and the mixture treated with a combination of Mörner's and Levene's methods, as indicated on page 344. The final product possessed all of the glucothionic acid properties of the substance of similar nature separated from the insoluble residue. All of the product was used for qualitative tests, so that no quantitative analyses could be made of it.

The above results indicate that the methods now in general use to separate the proteoses are inadequate for the removal of associated glucothionic acid, such as is broken from mucoid in peptic proteolysis,

<sup>1</sup> WITTE's peptone, in quantities equal to those of the muco-products used by us in these tests, and under similar conditions, gave no reduction of Fehling's solution either before or after decomposition.

or they show that the mucoproteoses separated by the usual methods from gastric digestive mixtures are substances of *variable* character, and that these variations are largely dependent upon the proportions of glucothionic acid radicles contained in, perhaps permanently combined with, these hydrolytic products. The qualities of the mucopeptones are more constant.

These deductions are in harmony with the analytic data tabulated on page 347.

The general conclusions on page 346, in connection with the indigestible matter, apply here equally well.

Further investigation will be directed to a more detailed study of the zymolytic products of glucoproteids of various kinds in both gastric and pancreatic fluids. These studies will be extended to such products as ovomucoid, salivary mucin and amyloid.

#### SUMMARY OF GENERAL CONCLUSIONS.

Connective tissue mucoids are digestible in pepsin-hydrochloric acid. The digestive process is relatively slow and gradual, however, and considerable substance remains insoluble even under the most favorable zymolytic conditions. In all of our experiments the residue amounted to at least 10 per cent of the original mucoid.

The soluble products are albuminate, primary mucoproteoses (proto-, hetero-, dysproteoses), secondary mucoproteoses (deutero-proteoses A, B, and C), and mucopeptones (peptones A, and B). The general properties of these bodies are identical with those of typical peptic products.

The indigestible matter appears to consist mainly, if not wholly, of resistant compounds of proteid and glucothionic acid. In most cases the mucoproteoses were also found to be glucothionic acid products of varying composition. It is possible, however, that the customary methods used in the isolation of the proteoses are inadequate for separating any associated glucothionic acid.

A glucothionic acid similar to chondroitin sulphuric acid was separated from both the indigestible matter and the proteoses. The peptones did not contain the glucothionic acid radicle. At least 25 per cent of the indigestible matter consisted of combined glucothionic acid.

Connective tissue mucoids are readily digested by trypsin in alkaline solution. Tryptophan, leucin, and tyrosin are produced from them in abundance.



## ON THE RELATION OF AUTOLYSIS TO PROTEID METABOLISM.

By H. GIDEON WELLS.

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THROUGH the work of Salkowski, Jacoby, and others, we have recently become acquainted with the occurrence within the cells of the animal body of ferments that are capable of digesting these cells under certain conditions. Furthermore, it has been shown that these autolytic ferments can be found not only in all the cells of the animal body, but also in yeasts, bacteria, and other vegetable cells of various sorts, whence it seems that autolysis is a property common to all cell forms. As this autolysis is a form of proteolysis, and is a constant property of all cells, it is not going far to assume that the autolytic ferments are important factors in the proteid metabolism of the cells, if not the chief factors. This idea also fits in very nicely with the development of ideas concerning the reversible action of enzymes, permitting the conception that the autolytic enzymes act to maintain a nitrogenous equilibrium within the cells, building up and breaking down proteids as circumstances require. The study of autolytic processes is too new, however, to have permitted the complete establishment of a relationship, much less an identity, between the autolytic ferments of the cells and the cell elements controlling normal proteid metabolism. A possible means of establishing this relationship was suggested by recent developments concerning the interaction of bodies that is sometimes required to produce an active ferment. Enterokinase, producing an active enzyme by its action upon trypsinogen, as shown by Pavloff, must be considered the starting point of these latter researches, although there were examples known before this time. The similarity of this sort of interaction with that observed in the immune sera made Pavloff's observation particularly timely and striking, and Delezenne has shown that there is actually a close resemblance between the formation of trypsin and the formation of hæmolytic substances in immune sera. Cohnheim's

work with the glycolytic ferments, showing that it may be necessary for an internal secretion of the pancreas to combine with an otherwise inactive constituent of the muscle to produce an active glycolytic ferment, indicates the possibility of interaction occurring through the medium of the blood-stream. It seemed desirable, therefore, to apply these ideas to proteid metabolism, and to see if substances known to modify proteid metabolism have an effect upon cell-autolysis.

As a favorable object, the thyroid was selected. It is well known that the thyroid has a marked effect upon nitrogenous metabolism. When the gland is removed, or is so diseased that its functional activity is decreased, the amount of nitrogen eliminated is much reduced, and the evidences of cell-activity are correspondingly decreased. Conversely, administration of thyroid glands or active preparations from them, causes an increase in elimination of nitrogen. An example of an opposite effect is afforded by the kidney. Removal of one kidney seems to lead to an increased activity of nitrogenous metabolism, so that the remaining kidney may eliminate more nitrogen than did the two originally. A natural inference in these two instances is that the thyroid produces a substance that in some way stimulates proteid metabolism, while the kidney produces something with an inhibitory effect.

An attempt was made to see if these effects could be attributed to an action of some constituent of the thyroid and kidney upon the autolytic ferments of the body. Three series of experiments were performed as follows: Dog livers were ground to a paste, and fifty grams of the material used for each experiment. In most instances the dogs were killed by bleeding. Extracts were made of thyroid, kidney, spleen, and liver, by grinding these organs in a similar manner, mixing the mass with  $\frac{2}{8}$  salt solution, and straining through a fine sieve. In each experiment the ground liver was placed in a 250 c.c. Erlenmeyer flask, and 100 c.c. of either water or  $\frac{2}{8}$  salt solution added. To this was added a quantity of the extract of the other organs, in quantity representing five grams of the original tissue. Ten c.c. of pure toluol was added to prevent decomposition, the flasks tightly stoppered, and placed in the incubator at 37°, for from one to three weeks, being agitated daily. When the experiment was to be closed, the flasks were provided with cotton stoppers, and placed in a steam sterilizer at 100° for one hour, to check autolysis in all flasks at the same time. Each series of experiments consisted of the following, either in duplicate or in triplicate: 1. liver with thyroid ex-

tract; 2. liver with kidney extract; 3. liver with spleen extract; 4. liver with liver extract (as a control to the first three); 5. liver alone, boiled before being placed in the incubator (to indicate the amount of autolysis in the other flasks). A few additional experiments were made, using thyroiodin and boiled thyroid extract, and the amount of autolysis in the extracts alone was also determined.

The amount of autolysis was ascertained by determining the amount of nitrogen present at the end of the experiment: (1) in a form coagulable by heat; (2) the amount present in a noncoagulable form, but precipitable by zinc sulphate in acid solution, and (3) the amount of nitrogen not thrown down in either of these ways. The analyses were made as follows: Each flask was placed in boiling water after its contents had been acidified slightly with acetic acid, and kept there thirty minutes. The coagulable substances were then collected on a folded filter, and washed with 500 c.c. of boiling water, slightly acidified with acetic acid. The residues were dried completely, removed from the filters, weighed, and two quantities between one and two grams each taken for analysis by the Kjeldahl-Gunning method. From these results was calculated the amount of "coagulable nitrogen." The filtrate was reduced to 100 c.c. on the water bath, saturated with zinc sulphate, and sulphuric acid added in quantity to make the solution 0.5 per cent. The precipitate was collected on as small a filter paper as possible, and washed with 200 c.c. of saturated zinc sulphate solution with 0.5 per cent sulphuric acid. This precipitate, termed "precipitable nitrogen," was analyzed, together with the filter paper.

The filtrate, containing "soluble nitrogen," was made up to 250 c.c. with distilled water, and the nitrogen determined in each of two portions of 25 c.c.

Because of accidents in the way of breaking of distilling flasks, etc., only one of the three series was determined completely, but the results in all three, except for the gaps, were practically identical, so that the figures will be given only for the completed series, in which the duration of autolysis was twenty-two days.

It is evident from these figures that there is no decided difference in the results obtained in the different experiments. The variations in the figures are not more than are to be expected in such experiments, and are not constantly in favor of any one of the sets of materials. If there were any real interaction among the organs demonstrable in this way, the differences in the results would prob-

Material.		Coagu- lable N.	Precipi- table N.	Soluble N.
		per cent	per cent	per cent
Thyroid ext. + liver . . . . .	Flask 1	42.3	2.9	54.8
	Flask 2	41.2	3.1	55.7
	Flask 3	39.4	2.8	57.8
Kidney ext. + liver . . . . .	Flask 1	42.0	3.9	54.1
	Flask 2	43.2	3.2	54.6
Spleen ext. + liver . . . . .	Flask 1	40.8	4.1	55.1
	Flask 2	46.6	3.5	49.9
Liver ext. + liver (control) . . . . .	Flask 1	43.8	3.8	52.4
	Flask 2	42.1	3.1	54.8
Boiled liver (no autolysis) . . . . .	Flask 1	90.4	4.2	5.4
	Flask 2	87.1	4.9	8.0

ably be very considerable. The results in all three of the series, which used materials obtained from different lots of dogs, agree so well with each other, as far as results could be obtained, that the conclusion seems warranted that in the manner in which this particular series of experiments was conducted, it cannot be shown that extracts of thyroid, kidney, and spleen have any decided effect upon the autolysis of the liver of the dog. Certainly there is no such marked effect as Cohnheim<sup>1</sup> found in the case of glycolysis by extracts of pancreas and muscle. However, it is possible that other experiments performed with other materials or in a different manner, might give different results.

In conclusion I desire to express my indebtedness to the Department of Chemistry of the University of Chicago for the facilities of its laboratories in performing the chemical portion of this research; and to Professor Julius Stieglitz for much kind assistance.

<sup>1</sup> COHNHEIM: *Zeitschrift für physiologische Chemie*, 1903, xxxix, p. 336.

## THE UTILIZATION OF VEGETABLE PROTEIDS BY THE ANIMAL ORGANISM.

BY ELBERT W. ROCKWOOD.

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THE experimental work on the digestibility of vegetable proteids has been carried out largely with mixed foods, or without attempting to purify the proteid, or to free it from the cellulose of the cell-walls. Thus Woroschiloff<sup>1</sup> compared the food values of meat and peas, making two series of trials on man with each. His conclusions, based upon the changes occurring in strength, as well as in the size and weight of the parts of the body, led him to believe that more energy was derived from the meat. Rutgers<sup>2</sup> gives the results of a ten-weeks experiment on himself and his wife for the purpose of comparing the value of animal and vegetable proteids. First, nitrogen equilibrium was attained on a mixed diet of meat, rice, milk, wheat-bread, and vegetables; then the meat, milk, and rice were replaced by a vegetable mixture containing equivalent amounts of proteids, fats, and carbohydrates. These were not analyzed; but their composition was calculated from published analyses, corrections being made for indigestible residues according to the data of Stützer and Rubner. With the mixed diet, 8.1 per cent of the total nitrogen ingested was excreted in the fæces; with the vegetable diet, 16 per cent to 23 per cent was so excreted, nitrogen equilibrium being again attained with no loss of body-weight. Rutgers concludes that the vegetable proteid has the same food value as that of animal origin. Avsitidiski<sup>3</sup> gives the result of a test conducted on inmates of a civil prison in five series of ten days each. A comparison was made between a vegetable and a mixed diet. The former consisted of bread, peas, buckwheat, macaroni, potatoes, rice, cabbage, butter, and sugar; the latter of

<sup>1</sup> WOROSCHILOFF: *Berliner klinische Wochenschrift*, 1873, x, p. 90.

<sup>2</sup> RUTGERS: *Zeitschrift für Biologie*, 1888, xxiv, p. 351.

<sup>3</sup> AVSITIDISKI: *Inaugural Dissertation*, St. Petersburg, 1889. Abstract in ATWATER and LANGWORTHY'S *Digest of Metabolism Experiments*, p. 25.

meat, bouillon, bread, butter, and sugar. In the former 15 per cent to 16 per cent of the nitrogen ingested was recovered in the fæces; in the latter, 7 per cent to 10 per cent.

Cramer<sup>1</sup> investigated the food of a vegetarian, finding that 21 per cent of the nitrogen of the peas, potatoes, coarse breads, fruits, milk, and eggs, of which the diet consisted, left the body in the fæces. In the case of another vegetarian whose food contained, besides fruits and oils, "Pumpernickel" and graham bread, Voit<sup>2</sup> ascertained that 41 per cent of the nitrogen was contained in the fæces. When exactly the same food, in kind and amount, was given to the laboratory servant, who was not a vegetarian, 42 per cent was thus excreted; the last subject was not able to maintain his weight upon this diet.

Rubner<sup>3</sup> made trials of the digestibility of a number of vegetable foods in man. They included maize, rice, potatoes, wheat and rye bread, peas, macaroni, cabbage, and carrots. The duration of each experiment was two or three days, and the substance under investigation formed the chief part of the food. In all cases, where the result is stated, there was a loss of body proteid. Of the nitrogen taken in with macaroni mixed with gluten, 11.2 per cent was found in the fæces; with the remaining foods the nitrogen thus eliminated amounted to from 17 to 39 per cent. In an additional series Rubner<sup>4</sup> states that from 20 to 30 per cent of the nitrogen of wheat flour appeared in the fæces, the utilization varying with the fineness of the flour, and being best with the very fine. Mori<sup>5</sup> quotes Osawa,<sup>6</sup> who made experiments on men. He found 59.3 per cent of the undigested proteid of boiled barley in the fæces; of boiled rice, 20.7 per cent; of Soja beans, 24.7 per cent; and of "Tofu," 3.9 per cent. This was compared with raw fish, which yielded from 2.0 to 2.3 per cent, and dried fish (cod and herring), from 4.7 to 7.1 per cent. According to Mori<sup>7</sup> the tofu is prepared by rubbing fresh Soja beans to a paste, bringing to a boil, and filtering when cold; from the filtrate the proteids are precipitated by the mother-liquor from the manufacture of salt, — a fluid containing much magnesium chloride. This

<sup>1</sup> CRAMER: *Zeitschrift für physiologische Chemie*, 1882, vi, p. 346.

<sup>2</sup> VOIT: *Zeitschrift für Biologie*, 1889, xxv, p. 232.

<sup>3</sup> RUBNER: *Zeitschrift für Biologie*, 1879, xv, p. 115.

<sup>4</sup> RUBNER: *Ibid.*, 1883, xix, p. 45.

<sup>5</sup> MORI: *Jahresbericht über die Fortschritte der Thierchemie*, 1892, p. 468.

<sup>6</sup> OSAWA: *Eiseikwai, Zasshi*, 1887, No. 48.

<sup>7</sup> MORI: *Zeitschrift für Biologie*, 1889, xxv, p. 102.

precipitate is filtered out, pressed, and eaten before fermentation has commenced. Mr. Mayesawa, a student at Yale University, states that in some parts of Japan the tofu is made by simply macerating the beans and pressing into a cake. Prausnitz<sup>1</sup> determined the faecal nitrogen from beans, boiled until soft, then cooked with butter and flour, as 30.3 per cent of the total content in the food.

Many investigators have studied the digestibility of the proteids of bread. Menicanti and Prausnitz<sup>2</sup> in eleven experiments with rye and wheat bread, using both that with and without the outer coatings of the grain, found that the faecal nitrogen was from 13.3 to 30.3 per cent of the nitrogen intake. Among the other studies are those of Jacoangeli and Bonani<sup>3</sup> who found that with the best grades of Italian breads from 4.9 to 17.8 per cent, and with the whole wheat breads, from 18.5 to 35.4 per cent of the ingested nitrogen was excreted in the faeces. Woods and Merrill<sup>4</sup> give as the percentages of faecal nitrogen for fine wheat bread, from 10.6 to 13.6 per cent; for whole wheat bread, from 13.2 to 13.3 per cent, and for graham bread, from 23 to 24.5 per cent of the food nitrogen.

Constaninidi<sup>5</sup> tried the utilization of the wheat gluten which is left as a by-product in the manufacture of starch, using dogs and men as subjects. To the former it was fed with lard, from 2.6 to 3.3 per cent of the nitrogen passing off in the faeces; for the men, the diet contained gluten, potatoes, and butter, 6.4 per cent of the nitrogen appearing in the faeces; whereas with the same diet, without the gluten, they contained 25 per cent. Leipziger,<sup>6</sup> in metabolism experiments on dogs with a diet composed of edestin (crystallized hemp-seed proteid), starch, and lard, and in addition, salts, meat extract, and water, found in two cases 3.9 per cent and 1.9 per cent of the ingested nitrogen in the faeces. Zadik,<sup>7</sup> with a similar diet, recovered 3.9 per cent and 3.4 per cent; and Ehrlich<sup>8</sup> obtained 3.6 per cent and 8.3 per cent of the ingested nitrogen in the faeces.

<sup>1</sup> PRAUSNITZ: *Zeitschrift für Biologie*, 1890, xxvi, p. 227.

<sup>2</sup> MENICANTI and PRAUSNITZ: *Ibid.*, 1895, xxx, p. 328.

<sup>3</sup> JACOANGELI and BONANI: *Jahresbericht über die Fortschritte der Thierchemie*, 1898, p. 628.

<sup>4</sup> WOODS and MERRILL: *Bulletin 85, Office of Experiment Stations*, 1900.

<sup>5</sup> CONSTANINIDI: *Zeitschrift für Biologie*, 1887, xxiii, p. 438.

<sup>6</sup> LEIPZIGER: *Archiv für die gesammte Physiologie*, 1899, lxxviii, p. 402.

<sup>7</sup> ZADIK: *Ibid.*, 1899, lxxvii, p. 1.

<sup>8</sup> EHRLICH: *Inaugural Dissertation*, Breslau, 1900.

Szumowski<sup>1</sup> tested the digestibility of zein (corn proteid) *in vitro*, and found that the crude substance was not much attacked by either pepsin or trypsin, but that 99 per cent of the purified zein was soluble in pepsin-hydrochloric acid and 95 per cent in trypsin solution.

Hammarsten<sup>2</sup> expresses the opinion that the vegetable proteids are in part indigestible; Moore<sup>3</sup> likewise says that they are less readily digestible than the animal proteids.

The results obtained by some of the above methods cannot be said to represent the real digestibility of the proteids, since this may be greatly modified by the other materials present, particularly by the cellulose. A few series of experiments were therefore carried out with the purified vegetable proteids, as well as some less pure preparations, to obtain further information regarding the utilization of the proteid and the conditions which affect it. The degree of utilization is assumed to be indicated by the difference between the nitrogen of the food and that excreted in the *fæces*. The fact is recognized that the latter, according to the investigations of Prausnitz,<sup>4</sup> contain much nitrogen from the epithelium of the alimentary tract and its secretions, as well as from the undigested food; yet it is believed that, keeping the other conditions as nearly constant as possible, this method of calculation at present furnishes the most satisfactory way for expressing the comparative digestibility (or availability) of the proteid food.

#### THE EFFECT OF COOKING ON THE DIGESTIBILITY OF OAT PROTEID.

**Experiments with dogs.**—Paton<sup>5</sup> noted that dogs failed to absorb from 14 to 55 per cent of the proteids of "oatmeal porridge." The latter was prepared by heating the oatmeal with two to three and one-half times its weight of milk, the duration of heating not being stated. The amount of unabsorbed nitrogen was determined by subtracting that of the urine from the total quantity in the food, no evidence being given that the animals were in nitrogen equilibrium. It seemed desirable to repeat this work in order to learn whether the small utilization could be explained on the ground of insufficient cooking of the oatmeal.

<sup>1</sup> SZUMOWSKI: *Zeitschrift für physiologische Chemie*, 1902, xxxvi, p. 198.

<sup>2</sup> HAMMARSTEN: *Lehrbuch der physiologischen Chemie*, 1899, p. 312.

<sup>3</sup> MOORE: SCHAEFER'S *Text-book of Physiology*: i. p. 442.

<sup>4</sup> PRAUSNITZ: *Zeitschrift für Biologie*, 1897, xxxv, p. 335.

<sup>5</sup> PATON: *Journal of physiology*, 1902, xxviii, p. 119.



Two series of experiments were carried out with dogs. In the first the coarse oatmeal was added to three times its weight of boiling milk, and the heating continued as long as the mixture could be conveniently stirred without burning, — about seven to eight minutes. By this process the grains of the oatmeal were, in large part, not disintegrated, and appeared again in the fæces. In the second series the same weight of oatmeal was ground as finely as possible with a coffee-grinder, steamed with three times its weight of water from four to six hours, mixed with the required quantity of milk, and evaporated until it became dry enough to be swallowed easily by the dog. The day's ration was given in the morning after catheterizing, washing out the bladder with sterile water, and weighing the animal. In no case was there any indication of fermentation in the bladder, the urine being constantly acid. Urine and washings were united, and the nitrogen determined by Kjeldahl's method in duplicate. The fæces were marked off by lampblack or fine sand, and dried on a water-bath after the addition of alcohol to produce a friable consistency and to aid in the evaporation of the water. A few drops of sulphuric acid were used to prevent the escape of ammonia, and, after mixing the air-dry fæces for the whole period, the nitrogen was determined in the same manner as in the urine. Mixed samples of each day's milk were preserved by formaldehyde, and the amount of nitrogen estimated at the end of the period. The food was readily eaten; the fæces were watery and appeared to contain much undigested material.

*Experiment A.* — A fox terrier bitch whose urine contained no albumin or proteose was used. The experiment extended over three dietary periods of six days each. The first was one with slightly cooked oatmeal; the second, one with well cooked oatmeal; and the third, with animal-proteid food. The fuel value of the food in the first two periods, as calculated from the usual composition,<sup>1</sup> was 468 calories; in the last period, it was 515 calories. The meat was chopped and preserved frozen, according to the method of Gies.<sup>2</sup> It contained 3.87 per cent of nitrogen. The dog's weight was 4.6 kilos throughout. The diet contained:

In the oatmeal periods.		In the meat period.	
Milk . . . . .	225 c.c.	Milk . . . . .	225 c.c.
Oatmeal . . . . .	75 gm.	Lean meat . . . . .	100 gm.
		Lard . . . . .	25 "

<sup>1</sup> ATWATER and BRYANT: Chemical Composition of American Food Materials, 1899.      <sup>2</sup> GIES: This journal, 1901, v, p. 235.

## SUMMARY OF OUTPUT AND INTAKE.

NITROGEN OUTPUT IN THE URINE.			
Day.	Slightly cooked oatmeal.	Well cooked oatmeal.	Meat diet.
	grams	grams	grams
1	2.54	3.04	4.00
2	....	2.64	4.44
3	2.74	2.77	4.58
4	....	2.76	4.47
5	2.61	....	4.71
6	2.61	3.00	4.68
Average daily output of N in the urine	2.63	2.84	4.48
Average daily weight of air-dry faeces . .	16.20	12.45	3.82
Average daily output of N in faeces . . .	0.613	0.498	0.258
Average daily intake of N in food . . .	3.11	3.14	5.11
Relation of faecal N to food N . . . . .	19.7%	15.8%	5.1%

In a food like that of the first two periods, where the only varying factor is the mode of preparation, we may get the value of the latter by assuming that the utilization of the milk would be the same in both periods, and that the difference in the amount of nitrogen in the faeces is due solely to the difference in the digestibility of the oat proteid. This difference, or "indigestible increment," as we may call it, can be obtained by subtracting the daily faecal nitrogen of one period from that of the other, and then finding the ratio of this difference to the daily nitrogen of the oatmeal. Thus, in Experiment A, we have for the daily difference in faecal nitrogen  $0.613 - 0.498 = 0.115$  gm. The nitrogen per day in the oatmeal was 1.887 gm. The ratio,  $\frac{0.115}{1.887} = 6.0$  per cent, represents the indigestible increment, or variable in digestibility, of the oat proteid due to the difference in cooking.

*Experiment B.* — A fox terrier bitch was fed, as in Experiment A, with oatmeal and milk, the cooking being short in one period and of long duration in

the other. The estimated fuel value was 784 calories. For comparison the experiment was concluded with a diet of animal food of an estimated fuel value of 793 calories. The body-weight gradually increased from 9.7 to 10.1 kilos during the trial. The food was :

In the oatmeal periods.		In the meat period.	
Oatmeal . . . . .	125 gm.	Lean meat . . . . .	200 gm.
Milk . . . . .	375 c.c.	Lard . . . . .	30 "
		Milk . . . . .	375 c.c.

SUMMARY OF OUTPUT AND INTAKE.

NITROGEN OUTPUT IN THE URINE.			
Day.	Slightly cooked oatmeal.	Well cooked oatmeal.	Meat diet.
	grams	grams	grams
1	3.10	2.33	5.16
2	3.08	2.33	6.02
3	2.80	3.02	6.73
4	3.09	2.60	
5	2.96	3.08	
6	2.70	2.79	
Average daily output of N in the urine	2.96	2.69	5.97
Average daily weight of air-dry fæces .	24.7	16.6	10.5
Average daily output of N in fæces . .	0.911	0.794	0.620
Average daily intake of N in food . . .	5.43	5.15	9.38
Relation of fæcal N to food N . . . . .	15.4%	14.4%	6.6%

The results obtained agree well with those of the preceding experiment. The indigestible increment, or variable due to cooking, was here 3.8 per cent. This indicates that the long boiling had very little influence on the utilization of the oatmeal. The analysis of the urine shows that this dog, unlike the one in Experiment A, was not in nitrogen equilibrium, but was storing a large proportion of the food nitrogen.

**Experiments with men.**— To learn whether similar results would be obtained with human beings whose digestive organs are perhaps better

fitted for a vegetarian diet than are those of dogs, two experiments were tried with men, one living on a vegetarian diet, the other eating meat as a part of his food. The kind and amount of the food was the same throughout the trial, but no determination of the total food nitrogen was made.

*Experiment C.* — The subject weighed, with clothing, 53.1 kilos at the opening of the series, and his weight remained constant. The daily diet was composed of:

Oatmeal . . . . .	75 gm.
"Shredded Wheat" Biscuit . . . . .	30 "
"Uneda" Biscuit . . . . .	180 "
Sugar . . . . .	100 "
Milk . . . . .	1350 c.c.

It had an estimated fuel value of 2536 calories. The method of cooking the oatmeal was the same as in the experiments with dogs, and it was eaten with the sugar and part of the milk at the noon meal. The first period of four days was that of slight cooking; the second, of three days, that with well-cooked oatmeal. Three days intervened between them.

#### UTILIZATION OF OATMEAL N. — SUMMARY.

	Slightly cooked oatmeal.	Well cooked oatmeal.
Average daily weight of air-dry fæces . .	27.5 gm.	26 2 gm.
Average daily weight of N in fæces . .	1.239 "	1.133 "

These figures show that the food was utilized better in the second period than in the first, but the indigestible increment does not differ materially from that obtained with dogs. It is 5.8 per cent, the weight of the ingested oatmeal nitrogen being 1.93 gm. per day.

*Experiment D.* — The body-weight was 66 kilos, with clothing. The diet consisted of:

Oatmeal . . . . .	100 gm.
Wheat bread . . . . .	75 "
Butter . . . . .	40 "
Hamburg steak . . . . .	400 "
Sweet potatoes . . . . .	300 "
Milk . . . . .	300 c.c.
Coffee with sugar . . . . .	1 cup

The oatmeal was not eaten at one time, but was divided between the three meals. There were two periods of three days each, during the first of which the oatmeal was cooked on a water-bath with 400 c.c. of water

for ten hours; in the second, it was heated in the same way for fifteen to twenty minutes. The estimated fuel value of the food was 2400 calories.

#### UTILIZATION OF OATMEAL N.—SUMMARY.

	Well cooked oatmeal.	Slightly cooked oatmeal.
Average daily weight of air-dry fæces . .	16.4 gm.	28.1 gm.
Average daily weight of N in fæces . .	1.05 "	1.78 "

As the nitrogen of the oatmeal amounted to 2.555 gm. daily, the indigestible increment in this experiment was 26.8 per cent.

#### CONCLUSIONS AS TO THE EFFECT OF THE DURATION OF COOKING ON THE DIGESTIBILITY OF OATMEAL.

All of the experiments show that the utilization of the proteid is increased by long cooking, and it is a natural supposition that this is the result of the breaking down of the cell-walls, and the easier access of the digestive enzymes to their contents. In the first three experiments, however, the increase is comparatively slight, even after grinding finely and long cooking. They probably give a more accurate representation than does the last one, where the diet was so complex. The fact that the last subject was unaccustomed to living upon a fixed diet, and found it monotonous, may have contributed to the poor utilization in the last part of the series. Neither of these conditions was true of the preceding subject. In Experiment D, the great increase in the fæces also indicates that the utilization during the last period was not as good as in the first. The air-dry fæces had been left for some hours exposed to the atmosphere, after a thorough drying on the water-bath. They would not contain exactly the same percentages of moisture, but it is believed that the difference would not be considerable. At least it could not cause such marked variations in their total weights.

The wide discrepancies between these results and some of Paton's may be explained on the theory that his dogs were not in nitrogen equilibrium. Indeed some of his figures point in this direction. He found (*loc. cit.* p. 119) with the same dog in three different series:

Series.	Oatmeal. grams	Milk. grams	Unabsorbed N. per cent
I	400	700	55
II	350	700	50
III	200	700	26

The unabsorbed proteid was found by subtracting the urinary nitrogen from that of the food. It seems probable that with an increase of the food beyond the actual needs of the body, more of the nitrogen was stored, although it was reckoned with the unabsorbed by this method of procedure. In the above experiments there was just such an apparent increase in the unabsorbed nitrogen running parallel to the increase in oatmeal. If the results in Experiment B, where the dog was not in nitrogen equilibrium, were calculated in this way, the undigested proteid would be from 45 to 48 per cent, which is, of course, incorrect.

#### THE COMPARATIVE DIGESTIBILITY OF PROTEIDS.

*Experiment E.* — The same bitch was used as in Experiment B. The experiment was divided into three periods: the first, of three days, with a diet of lean meat, lard, and cracker-dust; the second, of four days, with a diet of extracted proteid and milk; the third, of five days, with a diet of "Norka," or malted oats, and milk. The proteid of the second period was extracted from rolled oats by five to six times their weight of 0.2 per cent potassium hydrate at ordinary temperatures. The solution was filtered through straining-cloth, and the proteid precipitated by neutralizing with dilute acetic acid. The precipitate was allowed to stand for twenty-four hours in an equal volume of alcohol, then filtered off and dried on a water-bath. It contained much starch, the nitrogen content being only 2.84 per cent. The food was prepared by boiling this five to ten minutes with the milk. The cereal food — Norka — purports to be a "malted oat food." As its proteid content ( $N \times 6.25$ ) was found to be 16.06 per cent, and as it contains a considerable amount of a reducing sugar, this is probably a fair representation of its character. It was fed mixed with the milk, without heating, and was eaten greedily by the dog. The urine was collected and analyzed as before. The dog's weight was constant at 10.7 kilos. The food contained:

In the meat period.		In the oat proteid period.		In the Norka period.	
Lean meat	260 gm.	Oat proteid	} . 111 gm.	Norka	123 gm.
Lard	50 "	mixture		Milk	375 c.c.
Cracker-dust	60 "	Milk	375 c.c.		
Water	250 c.c.				

## SUMMARY OF OUTPUT AND INTAKE.

NITROGEN OUTPUT IN THE URINE.			
Day.	Meat period.	Oat proteid period.	Norka period.
	grams	grams	grams
1	5.53	3.91	3.54
2	6.30	3.73	3.71
3	6.93	3.60	3.75
4	....	3.44	3.85
5	....	....	3.74
Average daily output of N in urine . .	6.25	3.67	3.72
Average daily weight of air-dry fæces . .	7.50	4.35	12.07
Average daily output of N in fæces . .	0.456	0.238	0.433
Average daily intake of N in food . . .	10.62	5.44	5.44
Relation of fæcal N to food N . . . .	4.3%	4.4%	8.8%

*Experiment F.* — The animal was the one used in Experiment A. Her weight varied within the limits of 3.67 and 3.88 kilos during this trial. The urine and fæces were collected and analyzed as before. The experiment was divided into four periods. During the first seven days the animal was fed with an extracted oat proteid, the next six days with "Quaker Rolled Oats," the next four with a meat diet, and the last three with one which contained zein, an alcohol-soluble corn proteid.

The oat proteid of the first period was extracted as in the last experiment, but this time the starch was allowed to settle out of the liquid as far as possible before filtration, the supernatant liquid being decanted through the filter. By this means a dry substance containing 11.28 per cent of nitrogen was obtained. This powder was ground in a mortar, after thoroughly drying on the water-bath. It was boiled with starch and milk about five minutes, until it became too thick to stir without burning. The small particles of the dried proteid were then still plainly visible. It had a calculated fuel value of 475 calories. In the second period the fuel-value was kept the same, the rolled oats being used to ascertain whether the change of form affected the degree of utilization. The fuel value of the third period was calculated as 700 calories. The zein of the fourth

period was dissolved by alcohol from corn residues from which the starch had been largely removed. The alcoholic solution was precipitated by dilution with water, and the zein thus obtained was thoroughly dried at 100° after washing with water. It was very hard after drying, and was ground as finely as possible before feeding. It contained 11.99 per cent of nitrogen, showing that it was not pure. The food was boiled together until it thickened, when the yellow grains of zein were still visible. From the faeces, the zein could be recovered in considerable quantities by alcohol. The food contained:

In the oat proteid period.		In the rolled oats period.	
Oat proteid . . . . .	16.8 gm.	Rollled oats . . . . .	74.9 gm.
Starch . . . . .	60.0 "	Milk . . . . .	225.0 c.c.
Milk . . . . .	225.0 c.c.		
In the meat period.		In the zein period.	
Lean meat . . . . .	100 gm.	Zein . . . . .	15.8 gm.
Lard . . . . .	25 "	Lard . . . . .	10.0 "
Starch . . . . .	50 "	Starch . . . . .	50.0 "
Milk . . . . .	225 c.c.	Milk . . . . .	225.0 c.c.

## SUMMARY OF OUTPUT AND INTAKE.

NITROGEN OUTPUT IN THE URINE.				
Day.	Oat proteid period.	Rollod oats period.	Meat period.	Zein period.
	grams	grams	grams	grams
1	3.04	2.39	3.78	2.82
2	2.18	2.71	3.82	2.48
3	2.18	2.61	3.88	2.68
4	2.58	2.71	4.10	....
5	2.54	2.87	....	....
6	2.63	2.58	....	....
7	2.69	....	....	....
Average daily output of N in the urine	2.55	2.65	3.90	2.66
Average daily weight of air-dry faeces	6.80	10.80	7.40	14.80
Average daily output of N in faeces .	0.334	0.433	0.344	0.714
Average daily intake of N in food .	3.18	3.18	5.30	3.19
Relation of faecal N to food N . . .	10.5%	13.8%	6.5%	22.4%



*Experiment G.*—The same dog was used as in Experiments B and E. Her weight was constant at 9.3 kilos. Most of the nitrogen was given in the form of zein. This was fed in the moist condition, without being allowed to dry after precipitation. It contained 11.5 per cent of nitrogen. Comparatively large lumps of the undigested zein could be distinguished in the fæces. The food consisted of:

Zein . . . . .	90 gm.
Cracker-dust . . . . .	150 "
Lard . . . . .	50 "
Water . . . . .	225 c.c.

SUMMARY OF OUTPUT AND INTAKE.

NITROGEN OUTPUT IN THE URINE.	
Day.	Grams.
1	3.15
2	4.50
3	4.14
Average daily output of N in the urine	3.93
Average daily weight of air-dry fæces .	17.60
Average daily output of N in fæces . .	1.16
Average daily intake of N in food . .	11.23
Relation of fæcal N to food N . . . .	10.3%

CONCLUSIONS FROM DIGESTION EXPERIMENTS.

These may perhaps be more evident from a summary of the results. The table on the following page gives the percentages of the daily food nitrogen which were found in the fæces.

It is evident that without their removal from the materials associated with them the vegetable proteids are not utilized to the same extent as those of animal origin. The degree of utilization is somewhat, although only slightly, increased by long cooking. Rolling out into thin flakes, as in the rolled oats, also appears to have some effect; but oats thus treated yield nearly as much fæcal nitrogen as the others. The

## PERCENTAGES OF FOOD NITROGEN IN THE FÆCES.

Food.	Experiment.	Duration of cooking.	Nitrogen.
Meat, lard, and cracker-dust .	A	Uncooked	5.1
Meat, lard, and cracker-dust .	B	"	6.6
Meat, lard, and cracker-dust ..	E	"	4.3
Meat, lard, and cracker-dust .	F	"	6.5
Norka (malted oats) . . . . .	E	"	8.8
Coarse oatmeal . . . . .	A	5 to 8 minutes .	19.7
Coarse oatmeal . . . . .	B	"	15.4
Rolled oats . . . . .	F	"	13.8
Oat proteid . . . . .	E	"	4.4
Oat proteid . . . . .	F	"	10.5
Zein . . . . .	F	"	22.4
Zein . . . . .	G	"	10.5
Fine oatmeal . . . . .	A	4 to 6 hours	15.8
Fine oatmeal . . . . .	B	"	14.4

technical process employed in the preparation of the "malted oats" seems to favor utilization; and this may be explained by their partial hydration at that time. In E the extracted vegetable proteid showed itself to be capable of as great utilization as that of the animal proteids. That of F was less so. M. Voit,<sup>1</sup> in his investigations on the various forms of meat, found that in the form of dried powders they apparently suffer a considerable loss in their digestibility, which may be sufficient to explain the poorer utilization in F. In E, the large amount of starch presumably kept the proteid particles from drying together into a comparatively impervious mass. There is, consequently, no evidence from these experiments to indicate that the oat proteid, *per se*, is less readily utilized than is that of lean meat. This corresponds with the experience gained from feeding the vegetable proteid, edestin, already referred to.

It may be objected that the presence of the milk in the dietary vitiates the conclusions derived from these trials. In all the experi-

<sup>1</sup> M. VOIT: Zeitschrift für Biologie, 1904, xlv, p. 79.

ments with dogs (except with zein-feeding in G), the proportion of milk to the proteid studied was constant, and of the latter the same amount was used wherever practicable. Hence it is believed that the figures represent fairly the comparative degree to which the animals utilized the proteid. The greater faecal nitrogen from zein is probably not due so much to its being less digestible, as to the insolubility of the substance and the inability of the digestive enzymes to act vigorously, except upon the surface of the impervious masses. When the zein was thoroughly dried, its utilization was still further lessened. There is no reason to doubt that if the zein could be freed from the materials with which it is associated, except the starch, its utilization would be increased. The facilitating effect of the presence of starch was shown with the oat proteid in E. The achievement of means for removing the substances which interfere with digestion is worthy of study.

My thanks are due to Professor Lafayette B. Mendel for advice in the prosecution of this research.

THE INHIBITORY INFLUENCE OF POTASSIUM CHLORIDE  
ON THE HEART, AND THE EFFECT OF VARIA-  
TIONS OF TEMPERATURE UPON THIS  
INHIBITION AND UPON VAGUS  
INHIBITION.

By E. G. MARTIN.

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INTRODUCTION.

SINCE the time of Bernard it has been known that the cold-blooded ventricle is prevented from beating by the presence of potassium salts in the solution in which it is bathed. Ringer<sup>1</sup> noted that the addition of a small amount of potassium chloride to an alkaline sodium chloride solution in which a heart was beating well had the effect of arresting the ventricle. Bottazzi<sup>2</sup> emphasized the inhibitory nature of this potassium action, even going so far as to suggest its identity with vagus inhibition. Greene<sup>3</sup> considered the inhibitory action of potassium to be due to the tendency of this substance to produce relaxation of the heart-tissue. Howell,<sup>4</sup> while not going so far as Bottazzi, calls attention to the similarity between this potassium effect and other forms of inhibition. He also corroborates Greene's statement that potassium may exhibit a relaxing effect, although he is inclined to assign an even greater tendency in this direction to sodium. Stiles,<sup>5</sup> in a recent publication, showed that potassium in doses of less than 0.15 per cent causes a condition of diminished tone in strips of gastric tissue from the frog, although in greater concentrations it exhibits the reverse action.

Many workers have studied the effect of variations of temperature

<sup>1</sup> RINGER: *Journal of physiology*, 1883, iv, p. 38.

<sup>2</sup> BOTTAZZI: *Archives de physiologie*, 1896, p. 882.

<sup>3</sup> GREENE: *This journal*, 1898, ii, p. 121.

<sup>4</sup> HOWELL: *Ibid.*, 1901, vi, p. 205.

<sup>5</sup> STILES: *Ibid.*, 1903, viii, p. 271.

upon the cold-blooded heart, with the uniform result that up to a certain optimum, increase of temperature quickens the heart's action, while below this point lowering of the temperature is accompanied by slowing of the beat.

This study was undertaken at the suggestion and under the guidance of Dr. Howell, with a view to throwing additional light on the problem of potassium inhibition, especially with regard to the suggestion of Bottazzi that it may be of the same nature as vagus inhibition.

#### METHOD.

The isolated terrapin heart was used in the experiments, and was prepared so that the entire organ could be irrigated with nutrient solutions. The inflow cannula, which was inserted usually into the right vena cava, had a bulb blown upon it, and a side neck, admitting the stem of a small thermometer, so that the temperature of the circulating liquid could be read at the moment of its entrance into the heart. An arrangement was interposed in the circuit by means of which the temperature of the circulating medium could be varied at pleasure. The chlorides of sodium and potassium used in making up solutions were recrystallized from the chemically pure salts. As the ordinary fused calcium chloride cannot be used with safety in such experiments, and the unfused salt cannot be weighed with accuracy, an approximate solution was made, and the percentage of calcium then determined by analysis. All solutions were made up with distilled water. In preparing solutions containing various percentages of potassium chloride, care was always taken to keep the solution nearly isotonic with terrapin serum; therefore as the proportion of potassium chloride was increased, the proportion of sodium chloride in the solution was correspondingly diminished. The usual concentration of calcium chloride employed in making up Ringer's solution was used in every case. Since in these experiments the amount of calcium chloride employed did not exceed 0.025 per cent, the osmotic pressure of the solution was not sufficiently affected by the addition of the calcium chloride to change the physiological value of the solution so far as its osmotic relations were concerned.

#### THE POTASSIUM ARREST.

Although, as would be expected, there is considerable variation shown by different hearts in their reactions toward potassium chloride, the experiments were of such a nature that certain conclusions can be

drawn with regard to the arrest of the heart caused by this reagent. If we begin to perfuse a beating heart with Ringer's solution containing 0.03 per cent potassium chloride, the usual proportion employed in this laboratory, and increase the concentration of this salt 0.01 per cent at a time, the rate of beat usually diminishes gradually, and quite regularly, until the heart comes to rest. In one experiment the



FIGURE 1. — The effect of increasing concentrations of potassium chloride in the irrigating solution on the rate of beat. The ordinates represent the number of contractions in the minute. The numbers along the abscissa line represent the proportion of potassium chloride in the irrigating solution, expressed in hundredths of 1 per cent. Experiment of December 12, 1902.

point of stand-still of the ventricle was reached with a concentration of potassium chloride of 0.14 per cent. In another experiment of the same sort the ventricle continued active until the proportion of potassium chloride in the solution was increased to 0.26 per cent. Curves illustrating the effect of increasing the proportion of potassium chloride in the solution are given in Figs. 1 and 2.

If instead of increasing the percentage of potassium chloride in the solution gradually, we pass at once from normal Ringer's solution to one containing from 0.10 to 0.12 per cent potassium chloride, the arrest of the ventricle ensues with great promptness. The average time required for complete stand-still of the ventricle in six observations, made on three different hearts, was slightly less than two minutes. When still heavier doses of potassium chloride were suddenly applied to the beating ventricle, from 0.21 per cent upward,

the suddenness of the stoppage became very striking. In twenty-one observations, on five different hearts, the average time that elapsed between the application of the solution containing the large dose of potassium chloride, and complete stand-still of the ventricle, was one quarter of a minute, and in no one of these cases did the interval

between the introduction of the inhibiting solution and the cessation of activity exceed one-half minute. The concentration of potassium chloride used in these experiments varied from 0.21 per cent to 1.0 per cent. Of thirty-eight observations on the arrest produced by potassium chloride, in which percentages of this salt of 0.21 and upward were used, only one showed a period of as much as three minutes between the application of the inhibiting solution and complete arrest. The average period for the thirty-eight observations was eight-tenths minute. In each of these cases the heart was beating vigorously under normal Ringer's solution before the inhibiting solution was turned on.

The rapidity with which the arrest comes on is no greater when the inhibiting solution contains very high concentrations of potassium, 0.5 per cent and upward, than under potassium doses of from 0.25 per cent to 0.35 per cent. It would seem that under these smaller proportions of potassium the alteration in the tissue which produces stand-still, whatever that alteration may be, takes place as rapidly as the tissue is able to react, and hence a greater proportion of potassium in the solution cannot hasten the process. The potassium arrest can be obtained with a considerably smaller proportion of potassium salt in the solution if the heart is changed abruptly from a solution containing a small amount of potassium to one containing it in greater concentration, than if the change be made gradually by increasing the proportion of potassium a little at a time. In the former case arrest may be obtained frequently with from 0.08 per cent to 0.10 per cent potassium chloride, whereas by the latter method from 0.14 per cent to 0.16 per cent of the same salt will be required to effect this result. This fact agrees with a statement made by Howell<sup>1</sup> to the effect that heart tissue is able, to a certain

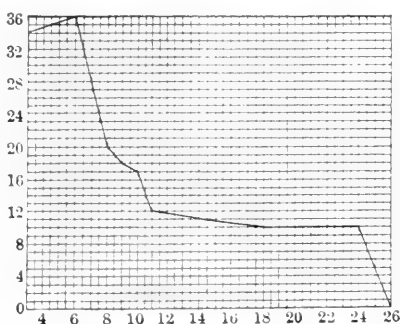


FIGURE 2. — The effect of increasing concentrations of potassium chloride in the irrigating solution on the rate of beat. The ordinates represent the number of contractions in the minute. The numbers along the abscissa line represent the proportion of potassium chloride in the irrigating solution, expressed in hundredths of 1 per cent. Experiment of October 16, 1902.

<sup>1</sup> HOWELL: This journal, 1901, vi, p. 202.

extent, to accustom itself to excessive doses of potassium salts, if these are introduced very gradually.

#### THE CONDITION OF THE HEART TISSUE DURING POTASSIUM ARREST.

Ringer<sup>1</sup> states that when the ventricle is inhibited by perfusion with potassium chloride (the strength is not stated), the muscle no longer responds to strong electric shocks. Bottazzi<sup>2</sup> says that the irritability of the heart toward electrical stimuli either diminishes enormously or disappears under potassium inhibition. Bottazzi, however, used a solution of potassium chloride, isotonic with the serum, for producing inhibition. Howell<sup>3</sup> refers to the statements of these authors, and adds the suggestion that probably, by graduating the dose of potassium chloride, a concentration would be found below which the ventricle can respond to stimuli, although not able to give spontaneous contractions. The following observations were made upon this point. Mechanical stimulation was used throughout, instead of electrical. It is not improbable that electrical stimulation would have given somewhat different limits of irritability. The first experiment was upon a heart which seemed to be unusually sluggish, as shown by the fact that a solution containing only 0.11 per cent potassium chloride kept it in complete inhibition for eighty minutes. During the time that the heart was at rest it was stimulated three times, at intervals of about fifteen minutes. The first stimulus was applied about forty-five minutes after inhibition had commenced. When the first stimulus was applied, the heart was at a temperature of 30° C.; the second stimulus was applied with the temperature at 20° C., and the third with the temperature at 15° C. In every instance the heart responded to the stimulation by a single well-marked contraction. In another case the inhibiting solution contained 0.33 per cent potassium chloride. Six minutes after the commencement of inhibition, with the temperature at 19.5° C., the heart responded to stimulation as before, by a single contraction for each stimulus. The same result was obtained from another heart, inhibited by a solution containing 0.28 per cent potassium chloride, with the temperature at 19° C. Another series of similar experiments was carried out, in which the solutions used to inhibit the heart

<sup>1</sup> RINGER: *Journal of physiology*, 1887, viii, p. 289.

<sup>2</sup> BOTTAZZI: *Loc. cit.*, p. 891.

<sup>3</sup> HOWELL: *Loc. cit.*, p. 205.



contained much larger proportions of potassium chloride. With the heart inhibited by a solution containing 0.5 per cent potassium chloride, stimulation, applied four minutes after the appearance of inhibition, caused a contraction. The same result was obtained from a heart under the influence of a solution containing 0.6 per cent potassium chloride, when the stimulus was applied four minutes after the beginning of inhibition, but response could not be obtained from this same heart eleven minutes after the onset of inhibition. When the inhibiting solution contained 0.7 per cent potassium chloride, the heart did not respond to mechanical stimulation four minutes after the beginning of inhibition. In none of these cases did the heart execute a single spontaneous contraction during the time that it was under the influence of the potassium-containing solution.

#### TONE VARIATIONS DUE TO POTASSIUM CHLORIDE.

Stiles,<sup>1</sup> in a paper already mentioned, showed that for stomach tissue the percentage of potassium chloride below which loss of tone might be expected is about 0.15 per cent. With higher doses, tonic contraction usually occurs. The author's experiments indicate that the same general fact, namely, a concentration of potassium chloride below which loss of tone, and above which tonic contraction may be expected, holds good also for the terrapin's heart, although the point at which neither relaxation nor tonic contraction occurs would seem, from an examination of all his records, to fall somewhat higher than the figure given by Stiles for the stomach of the frog. About 0.23 per cent potassium chloride appears to be the dose at which variations in tone do not occur in neither direction.

#### ESCAPE FROM POTASSIUM INHIBITION.

The first point to be noted under this head concerns the ability of the ventricle to break spontaneously through the inhibition caused by potassium chloride solutions. Considerable difference was noted in this respect according as the heart was fresh, that is, just isolated from the influence of the normal circulation, or had been for a long time subject to the action of inorganic solutions. In the latter case a larger dose of potassium chloride seemed to be required to maintain the heart in complete stand-still than when the organ had just been isolated. In one experiment a fresh heart was beating under normal

<sup>1</sup> STILES: *Loc. cit.*

Ringer's fluid at the rate of twenty-eight contractions in the minute, the temperature being  $19.5^{\circ}\text{C}$ . It was then irrigated continuously with a solution containing 0.1 per cent potassium chloride. It remained at rest for eight minutes, and then, in spite of the continuous action of the potassium-containing solution, began to beat again with a regular but very slow rhythm, not exceeding one contraction per minute. The temperature remained unchanged throughout the experiment. In another experiment a heart which had been continuously irrigated with inorganic solutions for two and one-half hours was brought to a stand-still from normal Ringer's solution by the introduction of a solution containing 0.25 per cent potassium chloride.

TABLE I.  
THE INFLUENCE OF HEAT UPON THE ESCAPE OF THE HEART FROM  
POTASSIUM INHIBITION.

Date. 1902.	Per cent KCl in inhibiting solution.	Temperature of inhibition C.	Temperature of recovery. C.	Rate per minute before inhibition.	Rate per minute after recovery.
Oct. 16	0.26	$20.0^{\circ}$	$27.5^{\circ}$	31	28
" 17	0.11	$18.0^{\circ}$	$33.0^{\circ}$	24	4
" 20	0.12	$21.5^{\circ}$	$28.0^{\circ}$	24	36
" 20	0.24	$22.5^{\circ}$	$30.0^{\circ}$	10	7
" 21	0.10	$21.0^{\circ}$	$29.0^{\circ}$	32	9
" 23	0.15	$14.5^{\circ}$	$26.0^{\circ}$	38	4

In five minutes it began to beat again slowly, and with less vigor than when fed with the normal solution. The temperature in this experiment was  $17^{\circ}\text{C}$ . In no case in which a heart escaped spontaneously from the inhibition brought about by the presence of potassium in the irrigating solution, did the beat approach the normal in rate.

A very different result was noted when the temperature of the inhibiting solution was raised. A number of records were obtained in which the beat was restored very well by the application of heat. Table I gives the results of six such observations made during the month of October, 1902.

Attempts to overcome the effects of potassium inhibition by heat were very rarely successful when the concentration of potassium in

the inhibiting solution exceeded 0.25 per cent, although almost uniformly so when the proportion of potassium was less than 0.25 per cent. This concentration of potassium is about the same as that which forms the usual upper limit at which spontaneous escape from potassium inhibition can occur.

A number of experiments were performed to determine whether the inhibitory effect of potassium can be directly counteracted by the addition of other substances to the inhibiting solution. The substances tested fall into the following five groups :

1. Substances which combine with potassium to form more or less insoluble compounds :

Gold chloride, platinum chloride, sodium tartrate, phosphoric acid.

2. Substances which are recognized as direct heart stimulants :

Caffein, strychnia, digitalin.

3. Substances which have been used on the cold-blooded heart to excite contractions :

Delphinin,<sup>1</sup> nicotine.

4. Substances which have been supposed to exert a favorable influence upon heart activity :

Lecithin, boric acid.

5. Various compounds of calcium :

Calcium chloride in solution stronger than physiological, calcium glycono-phosphate, gum arabic.

Those substances of this series which are recognized as proto-plasmic poisons were used in very dilute solution, so as to avoid injuring the heart permanently. No one of the substances tested was at all effective in restoring the activity of the heart during potassium arrest, although the fact that they had not caused permanent injury was shown by the normal series of beats which was subsequently given in every instance with a favorable circulating medium (normal Ringer's fluid). The concentration of potassium chloride used for inhibiting the heart in these experiments ranged from 0.25 to 0.40 per cent.

#### RECOVERY FROM POTASSIUM INHIBITION.

All experimenters who have studied the action of salts on the cold-blooded heart have noted the celerity with which the tissue resumes its normal function when changed from an inhibiting solution to one

<sup>1</sup> BOWDITCH: Arbeiten aus der physiologischen Anstalt zu Leipzig, 1871, p. 169.

favorable to activity. A somewhat extended study of the recovery which is obtained by washing out the inhibitory substance was made in connection with this series of experiments, inasmuch as previous experience had indicated that the only practicable method of overcoming the inhibitory influence of potassium must consist in removing the potassium from the tissue. In a previous paragraph attention was called to the surprising quickness with which the potassium effect shows itself; in this connection is to be noted the equally surprising rapidity with which the inhibition may be overcome. A number of tests showed that the small proportion of potassium chloride in normal Ringer's fluid has no appreciable effect in delaying the recovery from potassium inhibition, as compared with 0.7 per cent sodium chloride solution, or the same solution containing 0.025 per cent calcium chloride, without potassium chloride, and since the beat obtained from the whole heart with Ringer's fluid is superior to that given by it under the influence of the other solutions mentioned, Ringer's fluid was used exclusively in these observations.

It would appear, from *à priori* considerations, that the ease with which Ringer's solution is able to wash out the inhibiting potassium from the tissue should depend partly upon the concentration of potassium chloride which was used for producing the arrest, partly perhaps upon the length of time that the tissue has been under the influence of the potassium-containing solution, and there might also be a difference in the action of the Ringer's solution according as its temperature is high or low. In Table II, which gives the results of forty-five observations of the recovery from potassium inhibition by irrigation with Ringer's fluid, these factors are noted. The experiments recorded in this table were performed upon twelve different hearts.

Analysis of this table shows, in the first place, that the rapidity with which the potassium effect is overcome by irrigation with Ringer's fluid is about the same for all concentrations of potassium chloride up to 0.3 per cent. The average time required for the inhibition to be overcome, for the first twenty-four observations given in the table, in all of which the inhibiting solution contained not more than 0.30 per cent potassium chloride, was three minutes. When inhibition was caused by solutions containing higher concentrations of potassium chloride than 0.30 per cent, there was a marked lengthening of the time which elapsed between the commencement of irrigation with Ringer's fluid and the resumption of normal activity. The average time for seventeen observations (numbers twenty-five to forty-

TABLE II.

THE EFFECT OF RINGER'S SOLUTION IN OVERCOMING POTASSIUM INHIBITION.

No.	Date. 1902.	Per cent KCl used for inhibition.	Duration of inhibition in minutes.	Temperature of Ringer's solution. C.	Time for recovery in minutes.	Rate per minute before inhibition.	Rate per minute after recovery.
1	Oct. 24	0.12	7.0	21.5°	2.0	40	32
2	" 24	0.12	26.0	21.5	3.5	32	34
3	" 24	0.12	26.0	24.0°	1.0	40	36
4	" 28	0.12	27.0	20.0°	3.0	16	18
5	" 27	0.20	35.5	23.0°	6.0	24	18
6	Nov. 17	0.21	2.5	19.5°	2.0	18	18
7	" 18	0.21	4.0	19.0°	2.5	31	22
8	" 17	0.23	5.0	19.5°	2.0	18	18
9	Oct. 23	0.23	26.0	20.0°	3.5	38	34
10	" 24	0.24	22.0	22.0°	4.5	40	34
11	Nov. 21	0.25	2.0	24.0°	3.5	23	14
12	" 18	0.25	4.5	19.0°	5.0	23	19
13	" 17	0.25	11.5	19.5°	2.0	18	17
14	" 17	0.27	10.0	19.5°	4.5	19	17
15	" 18	0.28	14.0	19.0°	8.0	19	13
16	" 17	0.29	7.0	19.5°	8.0	18	10
17	Dec. 24	0.30	10.0	17.5°	2.5	28	25
18	" 24	0.30	10.0	17.5°	2.5	30	24
19	" 24	0.30	10.0	17.5°	3.0	23	21
20	" 24	0.30	10.0	17.5°	3.0	26	20
21	" 24	0.30	10.0	28.5°	1.5	28	30
22	" 24	0.30	10.0	26.5°	1.0	24	34
23	" 24	0.30	10.0	25.5°	1.5	24	32
24	" 24	0.30	10.0	25.5°	1.0	20	32
25	Nov. 17	0.31	8.0	19.5°	9.0	12	10

TABLE II—*continued.*

No.	Date, 1902.	Per cent KCl used for inhibition.	Duration of inhibition in minutes.	Temperature of Ringer's solution, C.	Time for recovery in minutes.	Rate per minute before inhibition.	Rate per minute after recovery.
26	Nov. 15	0.32	8.0	19.0°	10.0	14	13
27	" 17	0.33	9.0	19.5°	12.0	16	10
28	" 17	0.35	7.5	19.5°	19.5	21	10
29	" 18	0.35	10.0	19.0°	11.0	13	12
30	" 18	0.38	6.5	19.0°	12.0	12	16
31	" 18	0.41	6.0	19.0°	19.0	16	11
32	" 18	0.44	6.0	19.0°	21.0	20	23
33	" 18	0.47	7.5	19.0°	11.0	23	
34	" 20	0.50	5.0	25.0°	8.0	22	17
35	Dec. 27	0.50	10.0	15.5°	3.5	22	22
36	" 27	0.50	10.0	16.0°	7.0	38	13
37	" 27	0.50	10.0	17.0°	8.5	19	16
38	" 27	0.50	10.0	23.5°	2.0	22	38
39	" 27	0.50	10.0	28.0°	2.0	26	52
40	Nov. 20	0.50	20.0	25.0°	8.0	17	7
41	" 20	0.60	12.0	25.0°	8.0	15	12
42	" 20	0.70	34.0	25.0°	22.0	10	9
43	" 20	0.80	14.0	25.0°	20.0	11	5
44	" 21	1.00	3.0	24.0°	17.5	13	9
45	" 21	1.00	15.0	24.0°	40.0	8	14

one in the table) was nine minutes. The inhibition was caused in these cases by solutions containing from 0.31 per cent to 0.6 per cent potassium chloride. Four observations were made in which solutions containing potassium chloride in the highest possible concentrations compatible with the preservation of the normal osmotic relations of the tissue were used. The concentrations employed varied from 0.7 to 1.0 per cent. These observations are given in numbers forty-two to forty-five of the table. The average time between the commencement

of irrigation and the onset of activity in these four cases was twenty-five minutes. These results indicate that the changes wrought in the tissue by the presence of large percentages of potassium salts in the surrounding medium, are somewhat more deep-seated than when smaller doses of these salts are present, although the inhibitory effect of the potassium may be exerted by the smaller dose. Even the largest percentages of potassium chloride employed in these experiments did not seem to be permanently injurious to the heart tissue, inasmuch as the normal activity of the heart could be restored in every case, even after inhibition with the strongest solutions used, by washing out the excess of the potassium salt.

The length of time that the heart was under the influence of the potassium-containing solution did not seem to have much effect on the ease with which the inhibition could be overcome by irrigation with Ringer's fluid. If this factor entered into the case at all, it was in those instances in which the dose of potassium chloride was large. In some experiments in which high concentrations of potassium chloride were used for inhibiting the heart, there seemed to be a distinct lengthening of the time required for the overcoming of the inhibition, in those cases in which the duration of the potassium irrigation had been long, as compared with those in which it had been only momentary. It would appear from these results that the condition of the heart, during inhibition with light doses of potassium at any rate, is not one of progressive change, in which the heart sinks deeper and deeper under the effect of the salt, but that it remains in a quiescent, and so to speak permanent state under the potassium. Long irrigation with potassium-containing solutions of any concentration used in these experiments did not injure the tissue permanently, since it contracted normally in every case after removal of the inhibitory substance.

Two experiments were performed for the purpose of determining to what extent the temperature of the Ringer's solution used to overcome the inhibition of potassium, influences the rapidity with which this inhibition is overcome. The first of these experiments is recorded in numbers seventeen to twenty-four of Table II. Inhibition was caused by a solution containing 0.3 per cent potassium chloride in each observation of this experiment, and the duration of inhibition was ten minutes in every case. The average temperature of the Ringer's fluid in four of these observations was  $17.5^{\circ}\text{C}$ . The time required for recovery averaged 2.75 minutes. In four other observa-

tions the average temperature of the Ringer's solution was  $26.5^{\circ}\text{C}$ ., and the time for recovery averaged 1.25 minutes. The other experiment of this sort is recorded in numbers thirty-five to thirty-nine of the table. In these observations the heart was inhibited with a solution containing 0.5 per cent potassium chloride. Ten minutes was again the time of inhibition in every case. Three of the observations, in which the average temperature of the Ringer's solution was  $16^{\circ}\text{C}$ ., showed an average time of six minutes, as necessary for the heart to resume activity. The other two observations were made with Ringer's solution whose temperature averaged  $25.5^{\circ}\text{C}$ . The average time of recovery for these two was two minutes.

#### EFFECT OF VARIATIONS OF TEMPERATURE UPON THE RATE OF BEAT.

Cyon<sup>1</sup> made a careful study of the effect of variations of temperature upon the rate of the frog's heart-beat. He used rabbit's serum as a circulating medium, and found that when the frog's heart was perfused with this solution the rate of beat increased with the temperature until a certain optimum temperature was reached; above this temperature the rhythm of contraction became irregular, slowed rapidly, and the heart came to rest a few degrees above the temperature of greatest frequency of beats. When cooled down again, the heart resumed beating. According to Cyon's work, the low temperature limit of activity in the frog's heart lies near zero degrees C.

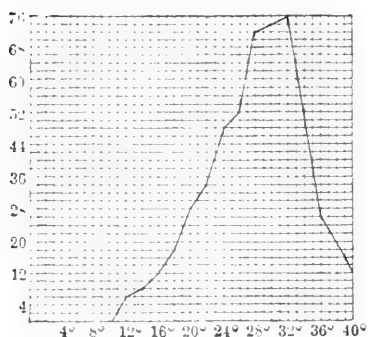


FIGURE 3. — The effect of variations of temperature upon the rate of the heart-beat. The ordinates represent the number of contractions in the minute. The figures along the abscissa line represent the temperatures. Heart perfused with normal Ringer's solution.

In connection with these experiments a study was made of the effect of variations of temperature upon the activity of terrapin's hearts, both

when irrigated with normal Ringer's fluid, and with solutions containing various proportions of potassium chloride. The low

<sup>1</sup> CYON: *Berichte der königliche sächsische Gesellschaft der Wissenschaften*, 1866, p. 257.



temperature limit of automaticity for the terrapin's heart appears to be somewhat higher than for the frog's heart. Frequently stand-still is observed under irrigation with Ringer's fluid at from  $8^{\circ}\text{C}$  to  $10^{\circ}\text{C}$ . The variations of rate with increase of temperature, as shown by this series of observations, agree closely with those recorded by Cyon, notwithstanding the fact that his experiments were upon a different animal and under slightly different circumstances. In one experiment, whose curve is given in Fig. 3, the ventricle was inactive at  $10^{\circ}\text{C}$ . As the temperature was raised beats began, and the rate increased quite uniformly with the rise in temperature until an optimum temperature,  $34^{\circ}\text{C}$ ., was reached. Above this temperature the rate fell off sharply, and the rhythm became very irregular. The point of complete stand-still was reached at about  $42^{\circ}\text{C}$ . In this experiment, as in all of the same kind performed by the author, it appeared that for any given heart the relation of temperature to rate was very nearly an absolute one. That is to say, whenever during the course of the experiment the temperature is at any point, the rate of beat at that time will be very nearly the same as at any other time when the temperature is the same. This is true whether the temperature is rising or falling at the time. In constructing the ascending limb of the curve in Fig. 3, the averages of four curves were taken. These four curves were closely parallel throughout their length. Two of them were curves of increasing temperatures, and two curves of decreasing temperatures.

An increase in the proportion of potassium chloride, too small to produce even temporary inhibition, has almost no effect upon the relation between temperature and rate. In Fig. 4 a curve is given to show this fact when the irrigating solution contained 0.08 per cent potassium chloride.

When the proportion of potassium chloride in the irrigating solution is large enough to produce temporary inhibition, followed by partial recovery, the form of curve obtained by plotting rates against

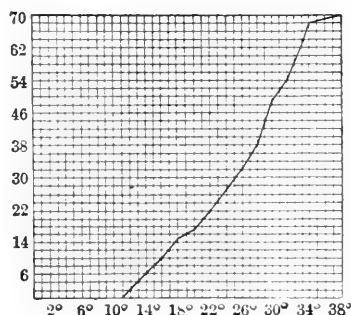


FIGURE 4. — The effect of variations of temperature upon the rate of the heart-beat. The ordinates represent the number of contractions in the minute. The figures along the abscissa line represent the temperatures. Heart perfused with a solution containing 0.08 per cent potassium chloride.

temperatures is, as would be expected, very different from the normal. In Fig. 5 a curve is shown in which the irrigating solution contained 0.11 per cent potassium chloride. The point of low temperature stand-still is much higher than normal, the point of optimal temperature is considerably lower than normal, and the high temperature limit occurs at a point about equivalent to the normal optimum. The curve as a whole is much flatter than those obtained when the heart is irrigated with solutions containing physiological amounts of potassium.

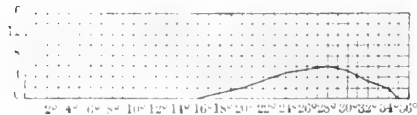


FIGURE 5. — The effect of variations of temperature upon the rate of the heart-beat. The ordinates represent the number of contractions in the minute. The figures along the abscissa line represent the temperatures. Heart perfused with a solution containing 0.11 per cent potassium chloride.

In a single experiment, the curve of which is given in Fig. 6, the heart was perfused with a solution containing sodium and calcium chlorides in the proportion in which they occur in normal Ringer's fluid, but containing no potassium salt. In this experiment the low temperature limit of automaticity was below that usually shown by hearts under the influence of Ringer's solution; in fact, with the method of cooling used in these experiments, the heart was not brought to rest by the lowest temperature obtainable. The increase in rate was more gradual than under corresponding conditions when Ringer's fluid was used for irrigating the organ, a fact one would scarcely anticipate when the inhibitory character of potassium is borne in mind. The optimum was reached at about the normal temperature, but the onset of heat stand-still was much more abrupt than in any other of the cases studied.

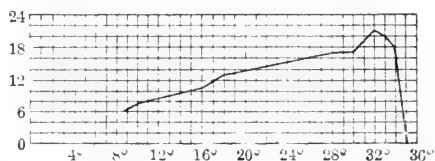


FIGURE 6. — The effect of variations of temperature upon the rate of the heart-beat. The ordinates represent the number of contractions in the minute. The figures along the abscissa line represent the temperatures. Heart perfused with a solution containing no potassium chloride.

THE INFLUENCE OF POTASSIUM CHLORIDE ON THE AURICLE.

Any one who studies the reactions of the heart to salts, cannot fail to be impressed with the great differences in these reactions exhibited by the different portions of the organ. Strips from the venous

end of the heart were studied with great care by Howell,<sup>1</sup> who showed that this tissue contracts spontaneously when immersed in a solution (Ringer's) containing a small proportion of potassium, although strips from the ventricle are completely inhibited by the same solution. The greater tolerance of the auricles toward potassium was strikingly illustrated in the series of experiments under consideration. One of the first effects of the application of a fairly concentrated solution of potassium chloride to the terrapin's heart, appears to be the severance of that consonance of rhythm which normally exists among the different chambers of the heart. The slowing of the ventricle, as the effect of perfusion with a potassium-containing solution, is always more marked than the corresponding slowing of the auricles. In fact, temporary inhibition of the ventricles may occur without any appreciable alteration in the auricular rate. In the experiment which is illustrated in Fig. 7, the ventricle came to rest under a solution containing 0.12 per cent potassium chloride, although under this same solution, at the same time, the auricles continued to beat vigorously at the rate of twenty-four contractions in the minute. In every case in which the ventricle escaped spontaneously from the inhibition of potassium, its rate after the escape was markedly slower than the corresponding beat of the auricles.

Fig. 7 illustrates the general effect upon the auricles of perfusing the heart with solutions containing gradually increasing percentages of potassium chloride. The curve fails, however, to show one interesting fact. In several instances the immediate effect of increasing the proportion of potassium chloride in the perfusing solution, was to depress the auricular rate to a point below that subsequently attained under the same dose of the potassium salt. In other words, the

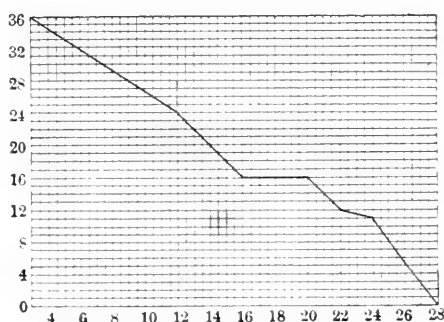


FIGURE 7. — The effect of increasing concentrations of potassium chloride in the irrigating solution on the rate of beat of the auricles. The ordinates represent the number of contractions in the minute. The figures along the abscissa line represent the proportion of potassium chloride in the irrigating solution, expressed in hundredths of 1 per cent.

<sup>1</sup> HOWELL: This journal, 1898, ii, pp. 56 *et seq.*

auricle can recover from the inhibitory action of potassium much more rapidly and effectively than can the ventricle. When we recall the greater thinness and delicacy of the auricular tissue, as compared with that of the ventricle, and that the former organ is presumably on that account much more permeable to salts, it becomes quite apparent that the reactions of the auricle to the various salts, especially to potassium, must be very different from those of the ventricle. From this standpoint, at least, it would seem that the two ends of the heart show independent properties. Another suggestive fact in connection with the effect of potassium upon the auricles is that the concentration of potassium chloride which the solution must contain in order to bring the auricles to rest is about 0.25 per cent. It will be remembered that this is just about the concentration which prevents the spontaneous escape of the ventricle, and which also is sufficient to maintain that part of the heart in entire quiescence in spite of the increased irritability which accompanies increase in the temperature of the organ. It seems probable that so long as the auricle continues beating, and discharging impulses into the ventricle, that part of the heart may escape from the inhibitory influence exerted upon it by the potassium in the solution, either spontaneously, or by the aid of heat, but when the inhibitory dose is large enough to bring the auricle to rest also, there is no longer a source of stimulation for the ventricle, and in the absence of such stimulation, the ventricle is held in complete inhibition.

#### EFFECTS OF VARIATIONS OF TEMPERATURE UPON THE AURICLES.

The auricle is affected in the same general way as the ventricle by variations in the temperature of the perfusing solution. The thinner texture of this organ renders it apparently more susceptible to variations of temperature than the heavier and thicker ventricle, but this greater susceptibility may be only apparent, and not real, simply because the thin auricle can more quickly attain the temperature of the surrounding solution than can the thick walled ventricle.

#### EFFECTS OF VARIATIONS OF TEMPERATURE UPON VAGUS INHIBITION.

G. N. Stewart<sup>1</sup> made a careful study of the effects of variations of temperature upon the action of the vagus in frogs. He did not perfuse his hearts, but immersed them in 0.6 per cent sodium chloride,

<sup>1</sup> G. N. STEWART: *Journal of physiology*, 1892, xiii, pp. 69, *et seq.*

whose temperature could be varied at pleasure. He states his results in the following words: (1) "If the temperature of the heart is lowered from the medium temperature, the inhibitory activity of the vagus is diminished, by whatever criterion that activity is estimated." (2) "As the temperature of the heart is increased from the medium temperature, the inhibitory action of the vagus is increased, whatever effect be taken as the test of its activity." After drawing these definite conclusions with regard to the effect of variations of temperature on the action of the vagus in the frog, he attempts to show that the same laws apply to the tortoise. He has this to say with regard to this point: "In general I find that the inhibitory action of the right vagus is affected by temperature in the same sense as that of the vagus in the frog; although the effect seems to be less marked than in the frog, and a much greater change of temperature is necessary to cause a sensible alteration in the inhibitory activity of the nerve. At very low temperature it is unquestionably more difficult to obtain complete stand-still of the heart than at the ordinary or at a higher temperature. But for a considerable range above and below the ordinary temperature it may be difficult to demonstrate any marked difference. It is by no means easy to show in the tortoise, what is seen in the frog, that the minimum strength of stimulus, needed to produce a given inhibitory effect, increases as the temperature falls and decreases as the temperature rises. It needs a considerable fall of temperature to appreciably increase the minimum stimulus." Results before Stewart's investigation were not concordant. Luchsinger and Ludwig,<sup>1</sup> working on the tortoise, arrived at the same conclusion which Stewart reached later, that cooling the heart diminishes the effect of the vagus. Lépine and Tridon,<sup>2</sup> on the other hand, stated that in the tortoise the action of the vagus persists when the heart is cooled, but is abolished when it is heated, returning again on cooling.

In view of the known effect of heat in increasing the heart-rate, presumably by accelerating the catabolic processes, the conclusions of Stewart seem unexpected. When the fact was brought out in the present series of experiments, that the inhibitory influence of potassium can be counteracted by heat to a considerable extent, the fact that the inhibitory action of the vagus is affected in precisely the opposite way by heat seemed so surprising that it was thought worth

<sup>1</sup> LUCHSINGER and LUDWIG: *Archiv für die gesammte Physiologie*, 1881, xxv, p. 211.

<sup>2</sup> LÉPINE and TRIDON: *Mémoires de la société de biologie*, 1876, p. 38.

while to repeat the experiments on the effect of heat on vagus inhibition, using the method of perfusion with inorganic solutions of various temperatures, in preference to the method of immersion in 0.6 per cent sodium chloride solution, which was employed by the earlier observers. No modification of the usual mode of isolating the heart *in situ* for perfusion was necessary, except that care had to be taken that the cardiac nerves were not included in any of the ligatures which were used in tying off the various vessels. A number of observations were taken, upon three different hearts. In every case the right vagus was stimulated as high up in the neck as convenient. The hearts were perfused with normal Ringer's solution, which, as is well known, maintains them in vigorous activity for very long periods. The experiments were of three sorts. In the first set of observations a strength of stimulus was selected which would hold the heart at a practical stand-still for several minutes at ordinary temperatures, and then the effect of raising and lowering the temperature was observed for this strength of stimulus. In the second set, advantage was taken of the fact that the heart of the terrapin can be maintained in stand-still by stimulation of the vagus for a number of minutes, without the effect of the stimulus becoming appreciably weaker. The heart was inhibited at ordinary temperature, and then while the stimulus was still on, the temperature of the organ was raised ten degrees or more, and before the stimulus was removed it was again cooled down to ordinary temperature. In the third set of observations a heart at room temperature was subjected to stimulation through the vagus with stimuli of gradually increasing intensity, and the point at which stand-still came on was noted. The temperature was then raised and the same procedure carried out. The point of stand-still at this temperature was compared with that at the lower temperature. It may be stated at once that in these experiments the inhibitory influence of the vagus was diminished by increase of temperature in every single instance, and that cooling the heart down again restored the inhibitory action to its former point. In detail the results of the experiments are as follows:

*Experiment of March 28, 1904.* — A heart was perfused with Ringer's solution, and the vagus was stimulated with tetanic shocks of varying intensity until a strength of stimulus was found which would just inhibit the heart. This occurred when the secondary coil was at a distance of nine centimetres from the primary. With the secondary in this position, and the heart beating vigorously and at the normal rate for the temperature, which

was  $20^{\circ}\text{C.}$ , the vagus was stimulated for three minutes. The heart gave two contractions during this time. The temperature was then raised to  $31^{\circ}\text{C.}$ , and the vagus was stimulated with the same strength of stimulation as before, for one and one-half minute. During this time it gave nine contractions. The temperature was again reduced, this time to  $20.5^{\circ}\text{C.}$ , and again the heart was subjected to the influence of vagus stimulation of the same intensity as before; the duration of the stimulation was three minutes, and the heart contracted once during this time. The secondary coil was then pushed one centimetre nearer to the primary, increasing the strength of the stimulus to a marked degree. The temperature of the heart was then raised to  $29^{\circ}\text{C.}$ , and the vagus was stimulated for three minutes. The heart gave nine contractions during the time the nerve was being stimulated. The temperature was then reduced to  $20^{\circ}\text{C.}$ , and the vagus stimulated for five minutes. The heart gave three contractions during the time of stimulation. The temperature was raised to  $29^{\circ}\text{C.}$ , and the vagus again stimulated for five minutes. The heart gave eleven contractions while the stimulus was on. Again the temperature was reduced, this time to  $19^{\circ}\text{C.}$ , and the vagus was stimulated for five minutes. The heart gave no contractions during the time of stimulation. Finally the temperature was raised once more to  $28^{\circ}\text{C.}$ , and the vagus stimulated again for five minutes. The heart gave thirteen beats during these five minutes. In every case the heart was beating regularly and rapidly when the stimulus was applied to the vagus nerve.

*Experiment of March 29, 1904.* — A heart was perfused with Ringer's fluid, and the minimal inhibitory stimulus through the vagus was determined. A different strength of current was used in operating the primary coil than in the experiment of the day before, and the secondary was placed at seven centimetres from the primary to give the stimulus desired. With the heart at  $21^{\circ}\text{C.}$ , the vagus was stimulated for two minutes. The heart contracted twice while the stimulus was on. The temperature was raised to  $29^{\circ}\text{C.}$ , and the vagus stimulated with the same strength of stimulus as before, for two minutes. The heart gave sixteen beats during the time the vagus was being stimulated. The strength of stimulus was increased by pushing the secondary coil one-half centimetre nearer the primary. The temperature was raised to  $31^{\circ}\text{C.}$ , and the vagus stimulated for one-half minute. The only noticeable effect of the stimulation was a slight slowing of the rate of beat, and a fall in the tone of the heart. The temperature was then lowered to  $22^{\circ}\text{C.}$ , and the vagus stimulated with the same stimulus as in the preceding case, for four minutes. The heart beat once while the stimulus was on. The temperature was again raised to  $30^{\circ}\text{C.}$ , and the vagus again stimulated, this time for three minutes. The heart contracted seventeen times during the stimulation.

*Experiment of March 30, 1904.* — A heart was perfused with Ringer's solution, and the minimal strength of stimulation of the vagus necessary to cause stand-still of the heart was determined. This occurred with the secondary coil at eight centimetres from the primary. With the heart at  $20^{\circ}$  C., the vagus was stimulated for three minutes. The heart contracted six times while the stimulus was on. The temperature was then raised to  $31.5^{\circ}$  C., and the vagus again stimulated for three minutes. The heart contracted thirty-nine times during this stimulation. The temperature was reduced to  $21^{\circ}$  C., and the vagus again stimulated. The heart gave no contractions during the three minutes of stimulation. The secondary coil was then pushed one centimetre nearer the primary, and the vagus stimulated for three minutes with the temperature of the heart at  $21^{\circ}$  C. No contractions were given while the stimulus was on. The temperature was then raised to  $31^{\circ}$  C., and the vagus stimulated for one minute. The heart gave thirteen beats in this minute of stimulation. The temperature was reduced to  $20.5^{\circ}$  C., and the vagus stimulated for four minutes. The heart was held throughout the time of stimulation in perfect quiescence. When the temperature was raised to  $29^{\circ}$  C., and the vagus stimulated for four minutes, the heart gave twenty-one contractions while the stimulus was on. Subsequent lowering of the temperature to  $21^{\circ}$  C., and stimulation of the vagus, inhibited the heart as completely as before under like conditions. The secondary coil was pushed still another centimetre nearer the primary, and the vagus was stimulated at  $21.5^{\circ}$  C., with the result that the heart was held in quiescence for four minutes. The temperature was then raised to  $32.5^{\circ}$  C., and the vagus stimulated for three minutes. The heart beat fifty-one times during the stimulation. When the temperature was again lowered to  $22^{\circ}$  C., vagus inhibition was again complete.

Two experiments were performed in which the temperature was raised during the stimulation of the vagus, and the influence of increase of temperature under this condition was determined. In each case a strength of stimulus was employed which was considerably greater than the minimum inhibitory stimulus, so as to insure that there could be no question of spontaneous escape from the inhibition, aside from that due to the heating. The two observations were made on two different hearts. In the first case the vagus was stimulated with the temperature of the heart at  $21^{\circ}$  C. After the heart had been under the influence of the inhibitory action of the vagus for two minutes, warm Ringer's fluid was turned into it. When its temperature reached  $27^{\circ}$  C., contractions began, and nine beats took place in two minutes. Meanwhile the warm solution had been turned off, and fluid of room temperature was perfused. As the temper-



ature of the heart fell below  $27^{\circ}\text{C}$ ., the organ again came to rest and remained so for two minutes longer, when the inhibitory stimulus was removed. In the second case, the inhibition was effected with the temperature of the heart at  $22^{\circ}\text{C}$ . After three minutes of quiescence the warm fluid was introduced, and again beats commenced when the temperature reached  $27^{\circ}\text{C}$ . In this case the rate of contraction became so rapid that the individual beats cannot be separated on the record, a slow drum having been used in these experiments. After three minutes of this rapid beating, the cool solution was turned into the heart, and as the temperature fell below  $27^{\circ}\text{C}$ ., the heart again came to rest, remaining so during the four minutes that the situation of the vagus was continued.

One attempt was made to compare the minimal inhibitory stimulus at room temperature with that at higher temperatures. With the heart at  $22^{\circ}\text{C}$ ., the vagus was stimulated with gradually increasing intensity, beginning with a sub-minimal stimulus. Pronounced slowing of rate occurred when the secondary coil was ten centimetres from the primary, and quiescence came on with it at a distance of nine centimetres. The temperature was then raised to  $31.5^{\circ}\text{C}$ ., and the vagus was again stimulated with increasing intensity, beginning with the secondary coil at the point which sufficed to inhibit the heart completely at the lower temperature. With the heart at this high temperature, no inhibitory effect was apparent with the secondary at this point, nor could the heart be brought to rest by the strongest stimulus which could safely be employed. The secondary was pushed to within three centimetres of the primary coil. To make sure that the nerve had not been injured, the temperature was again reduced to  $23^{\circ}\text{C}$ ., and increasing stimuli were again thrown into the nerve. This time the heart came to rest with the secondary coil at six centimetres from the primary.

These experiments seem to show conclusively that in the terrapin heart, perfused with favorable inorganic solutions, the inhibitory action of the vagus nerve is much more pronounced at room temperatures than at temperatures ten degrees above these.

#### CONCLUSIONS.

At the beginning of this paper the object in view was stated to be a comparative study of potassium inhibition and vagus inhibition, in order to determine, if possible, how much probability there may be in the suggestion of their identity. The two forms of inhibition have

the following points of similarity in the terrapin. Both can be so adjusted as to cause either a slowing of the heart rate only, or complete stand-still. The intensity of each can be so regulated that the heart retains its irritability throughout the inhibition. Stimulation of the vagus causes a diminution in the force of contraction, as well as in rate of beat. In the author's curves a similar diminution of force is to be seen in those cases in which the percentage of potassium chloride in the irrigating solution was the largest which the heart could bear without coming to rest. Smaller doses of potassium chloride did not have any appreciable influence on the force of the individual contractions. Both vagus and potassium chloride inhibition are counteracted by heat. In both forms of inhibition the effect becomes manifest very soon after the application of the inhibitory influence, and disappears promptly upon removal of that influence. Neither has marked after-effect in the terrapin. Relaxation of tone results from stimulation of the vagus, and also from irrigation with potassium chloride, provided the dose be not excessive. What appears at first sight to be a fundamental difference in the action of the two forms of inhibition is the fact that potassium inhibition affects the ventricle before it does the auricle, whereas the opposite is true of vagus inhibition. MacWilliam<sup>1</sup> has shown, however, that the relative responsiveness of the different parts of the heart to vagus stimulation depends rather upon the distribution of the inhibitory nerves in the organ than upon any greater intrinsic sensitiveness of one part as compared with another. He shows, also, that in the tortoise the inhibitory fibres are confined to the venous end of the heart. All researches into the effects of salts on cardiac tissue seem to show that the ventricle is more responsive to variations in the salt-content of the irrigating solution than is the tissue at the venous end of the heart. This fact is very apparent in the case of potassium, but it does not necessarily argue a fundamental difference between the inhibition brought about by this substance and that resulting from stimulation of the vagus nerve, since the distribution of the nerve is to those parts which are least sensitive to the salts. Both forms of inhibition act, probably, directly upon the muscle tissue; their influence upon it seems to be, in general, the same. Since in the terrapin the vagus effect is confined to the sinus and auricles, a true comparison between it and the potassium effect can only be made by considering those

<sup>1</sup> MACWILLIAM: *Journal of physiology*, 1885, vi, p. 224. See also GASKELL: SCHÄFER'S *Text-book of Physiology*, ii, p. 214.

cases of potassium inhibition in which the auricles were brought to rest. This result was obtained, it will be remembered, when the concentration of potassium chloride in the solution was about 0.25 per cent. Reference to the data given in this paper shows that there is a rather narrow range of concentrations of potassium chloride, at which the auricle may be inhibited, and yet those points in which potassium inhibition resembles vagus inhibition, namely, the phenomena of recovery by heat, loss of tone, retention of irritability, and prompt recovery from the inhibition, may all be present. The analogy, then, so far as these points are concerned, holds as well for the auricle as for the whole heart. These analogies seem to lend color to the suggestion that vagus inhibition and potassium inhibition may be identical, although they cannot be looked upon as a demonstration of this identity.

# THE HOURLY VARIATIONS IN THE QUANTITY OF HÆMOGLOBIN AND IN THE NUMBER OF THE CORPUSCLES IN HUMAN BLOOD.

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THE following observations include a study of the daily changes found in the peripheral blood of the healthy individual, and confirmed by previous studies of the blood-changes in dementia paralytica.

Reinert has shown that in the early part of the day the number of erythrocytes (or red cells) is comparatively high, and that after each meal there is a fall which becomes especially noticeable after the evening meal. The curve also falls gradually during the afternoon; but when the night rest begins the curve slowly rises, reaching the highest point at two o'clock in the morning. The hæmoglobin curve approximates closely to that of the number of the red cells, the minor differences which appear serving well to illustrate the dragging tendency of the hæmoglobin. The curve of the leucocytes rises gradually during the day up to 6 P. M., falls until 10 P. M., rises again until 12 A. M., and then falls to its lowest point at 6 A. M.

In the present study four healthy men were employed. Special care was taken to insure uniform conditions, and no change was made in the individual's daily occupation or habits. Complete records were kept of the weight, food, exercise, mental, and physical condition of each subject. The examinations were made as follows: in two cases the consecutive counts were carried through one day; continued in two other cases for two days each; and in another case for three days. Taken together, the examinations amounted to nine days' counting. This method possesses certain advantages over consecutive counts upon one person only, in that the individual equation becomes eliminated in the general averages.

It seemed advisable to precede each whole day count by at least

two check counts made at the same hour, usually before breakfast, and under identical conditions. For the whole day count itself, the periods were as follows: 7 A. M., 8 A. M., 11 A. M., 2 P. M., 5 P. M., 8 P. M., 10 P. M., 12 P. M. A single count was always taken on the next morning after the whole day count, serving as another check. This selection of examination periods was based upon Reinert's curves, as being the nodal or critical points of the blood changes of each day. In the first examination a count was made at every hour, but the nervousness of the subject, together with the fact that three persons were required to carry on the examinations, made it necessary to have but one person make the counts, and only at such times as appeared to be crucial.

Each count included estimations of the number of red and white blood-corpuscles, determination of the hæmoglobin, and estimations of the varieties of the white blood-corpuscles. In the estimations of the red corpuscles the Thoma-Zeiss instruments were used, and from one to two thousand cells were actually counted. Two instruments (Fleischel's) were employed to record the hæmoglobin. In the qualitative or differential work, each percentage was based on a count of from ten to twenty-five hundred cells. Two and three slides were used, and Jenner's stain preferred for the most part. A separate stab was often made to secure the blood for each of the pipettes and for the hæmoglobin estimation, thereby helping to eliminate certain errors of technique. Where there seemed to be any liability to error, the count was immediately repeated.

In Table I may be found the complete record of one day's observations, together with the check counts as made upon a single case. It includes the number of red and white blood-corpuscles, the percentage of hæmoglobin, and of the differential counts. With this brief mention of the conditions under which the experiments were carried on, we may now turn our attention to the results.

When we consider the final general averages which are graphically presented in Fig. 1, we find not only daily variations, but even hourly changes are present. The curves should be thought of as representing only the surface, as it were, of the volume of the blood.

The upper curve represents the variations in the number of the red cells. In the morning, the number of corpuscles stands a little above 4,900,000 per cubic millimetre, but as the day progresses this decreases gradually until the five o'clock period is reached, when the number stands at 4,650,000. Here, in less than twelve hours, there



is to be found a difference of 250,000 cells per cubic millimetre, or 5 per cent of the total number. From the five o'clock period, represented by the lowest point of the curve, there follows a steady increase culminating about three o'clock in the morning, from which time the falling curve of the next day apparently begins. The points plotted at the three and five (A. M.) o'clock periods represent only the figures obtained in one case, but in this case they held the same

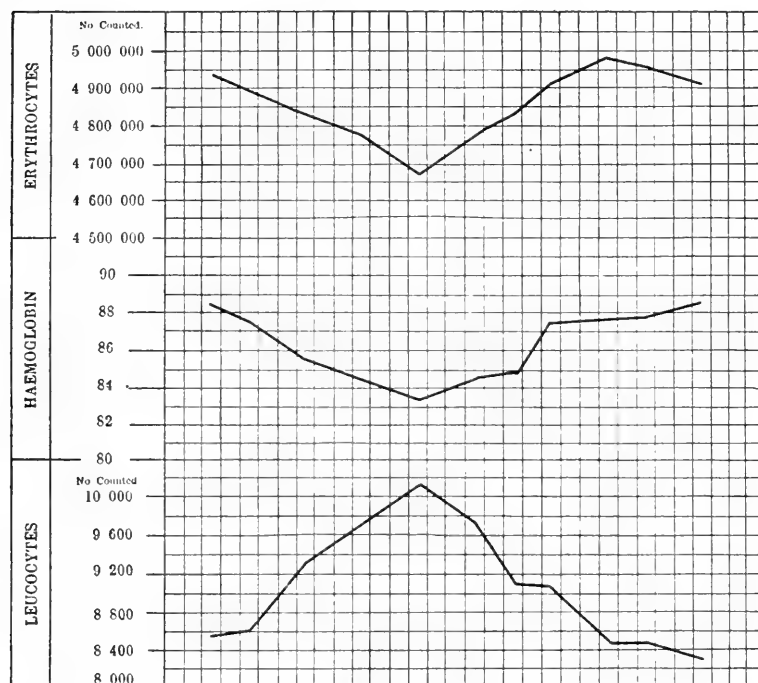


FIGURE 1.—Daily variations in the number of red and white cells per cubic millimetre, and in the percentage of hæmoglobin in the blood. Curves plotted from nine series of daily counts, of which one is given in Table I. The ordinates are hours, beginning at 6 A. M. on one day, and ending at 9 A. M. on the following day.

relative position in which they are here placed. Comparing these general changes found in the number of the red corpuscles, with those evidenced in the individual changes, we find that in every case the same conditions can be seen to exist.

The second curve represents the hæmoglobin changes. These follow those of the red corpuscles, step by step. The percentage stands highest in the morning at 88 per cent, and swings through a

difference of 5 per cent to its lowest point of 83 per cent, in the late afternoon. The estimation of the hæmoglobin is more open to error than any other part of the work, and for this reason gives the least satisfaction. Each reading taken by the operator varies slightly, as do the instruments. The important fact which should be borne in mind is that the *relative* and not the *absolute* values are to be considered.

In the leucocyte curve there is present a rather interesting series of changes. These same general changes are to be found in every instance of the eight days' estimations, and this evenness of results represents therefore not a mean of large differences, but a mean of

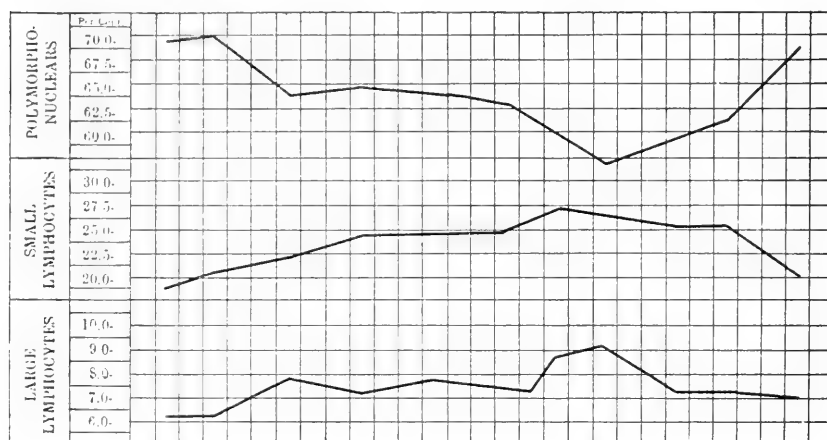


FIGURE 2.—Curves showing the daily changes in the relative percentages of the polymorphonuclear leucocytes and of the large and small lymphocytes. The ordinates represent hours, beginning at 5 A. M. on one day, and ending at 9 A. M. the following day.

remarkably small differences. The first period of the day begins with about 8,500 corpuscles per cubic millimetre. Every successive period where there was an estimation made, up to five o'clock (P. M.), shows that the number of cells is increasing. From then until the early morning hours there immediately follows a steady fall. The number of corpuscles ranges from 8,400 to 10,000 per cubic millimetre of blood, amounting to a difference of 1,600 cells, or about 20 per cent.

Probably the first thing to be seen in comparing the curves of the red and white cells is the apparent correlation of the changes which the two classes undergo. One must be careful for obvious reasons



not to draw erroneous conclusions that there is a compensation per volume. The same cause which diminishes the red corpuscles evidently increases the whites, a conclusion which would become evident in a rather striking way if the curve of the white corpuscles were super-imposed upon that of the red. This correlation is more apparent where the extremes of the blood-changes rest.

In the study of the differential counts various regular changes, similar to those just described, are found to occur. Fig. 2 contains the curves of the polymorphonuclear cells and lymphocytes divided into the large and small forms. These show definite and radical changes.

The polymorphonuclear cells comprise about 70 per cent of white cells in the morning, and fall off in percentage very slowly during the day, reaching the lowest point of 58 per cent about twelve o'clock at night. But from then until eight o'clock the next morning they rise again to 70 per cent. Mention might be made that each point of the curve is based on the recognition of some ten thousand leucocytes, *i. e.*, that the figures 70, 65, etc., stand for that per cent of ten thousand cells.

The small lymphocytes comprise about 20 per cent of the white cells at the first periods, and increase slowly, ranging up to 28 per cent at 12 P. M. Then there is a fall, and on the following morning approximately the same percentage is to be found. The large lymphocytes, constituting only a small percentage, still indicate the same tendency as the small lymphocytes. They range from 6-9 per cent. As in the case of the red and white corpuscles, we find here a definite relation between these two classes (the polymorphonuclears and the lymphocytes). That which lowers the percentage of one class increases the percentage of the other.

The eosinophiles and the mast cells are so few in number that they are of relatively little importance. In these observations they are present in about the same numbers throughout the day. Table II contains the final averages of the differential counts.

In addition to the estimations of the varieties which are usually included in the differential work, an attempt was made to estimate the percentage of disintegrating cells of the polymorphonuclears, eosinophiles, and the lymphocytes. Every well-stained film of blood shows these fragmentary cells, and in all the estimations they have been included. This attempt to answer the question what per cent of a single class of white cells are breaking up, has revealed one or two rather interesting facts.

TABLE II.  
FINAL AVERAGES OF THE DIFFERENTIAL COUNTS.

Classes.	Hour.										Aver- age.	
	6.30	8.00	11.00	2.00	5.00	8.00	10.00	12.00	3.00	5.00		8.00
Polymorphonuclears . . .	69.0	69.6	63.4	64.3	63.5	62.6	60.0	58.0	61.0	62.0	68.8	63.8
Small lymphocytes . . .	19.3	20.7	21.9	24.5	24.3	24.2	27.2	27.8	25.0	25.0	20.0	23.6
Large lymphocytes . . .	6.3	6.3	7.8	7.2	7.7	7.4	8.8	9.1	7.3	7.3	7.0	7.4
Eosinophiles . . . . .	3.6	2.7	3.4	3.0	3.2	3.8	3.5	4.3	3.3	4.0	3.4	3.4
Mast cells . . . . .	0.6	0.5	0.35	0.6	0.7	0.5	0.4	0.6	0.4	0.5	0.4	0.5
Disintegrating polynuclears	3.0	2.0	2.0	3.0	3.0	2.0	3.0	5.0	—	—	3.0	3.0
Disintegrating eosinophiles	5.0	2.0	3.0	4.7	2.0	4.0	7.0	4.2	—	—	—	4.0
Disintegrating lymphocytes	9.0	6.0	5.0	9.0	7.0	7.0	7.0	10.0	—	8.0	7.0	8.3

The polymorphonuclear cells which were breaking up amounted to three per cent. They were easily recognized by the achromatic condition of the nucleus, their broken contours, and by the size of the scattered granules. No conclusions can be drawn concerning the percentage of such cells as related to the daily changes. Four per cent of the eosinophiles were found to be disintegrating, though in the usual single blood-count one might easily believe that the percentage would be higher, so easily recognized are the cells. Exceeding both the polymorphonuclears and the eosinophiles in the percentage of cells breaking up (old cells), are the lymphocytes, especially the large forms, having an average of over 8 per cent. This is a rather interesting fact, in that it seems to show that of the cellular debris which exists in the blood-plasma at any one time at least 50 per cent comes from the lymphocytes.

In addition to the foregoing observations, a few notes were made upon the morphological appearance of some of the cells. Only brief mention will be made of these, since the facts are, for the most part, already accepted.

It has been observed that the eosinophiles commonly possess bilobed nuclei. A count was kept of such cells, and they were found to constitute 83 per cent of the total number of eosinophiles, the remaining 17 per cent possessing a trilobed nucleus. Special notice was taken of the minute finger-like processes often observable on the lymphocytes, especially of the small forms. Small lymphocytes which exhibited these processes amounted to 20 per cent. In this estimation were included none of the lymphocytes already designated as breaking up.

In two days' estimations, the percentage of lymphocytes which possess granules was determined and found to be about 12 per cent. There is room for questioning the fact whether these granules constitute any strictly morphological part of the cells. A few mast cells were found which possessed granules approaching in size those of the polymorphonuclear cells, and suggesting a transitional stage between the polymorphonuclear and the mast cells. Not a single myelocyte was seen, and only seven normoblasts during the recognition of over 80,000 leucocytes.

A series of hourly counts was made in a typical case of dementia paralytica, and the observations, which lasted through two days, confirmed in nearly every point the hourly variations as found in the present charts. In addition, a careful analysis of some four hundred

systematic blood-counts made upon individuals suffering from this same disease also confirmed the general facts of the preceding observations, for in one hundred and eighty-five morning counts the average number of red cells was high, — five million; in one hundred and fifty afternoon counts the average number of red cells was low, four million seven hundred thousand. In the case of the white cells the average morning count amounted to seven thousand two hundred cells, with eight thousand for the afternoon. The hæmoglobin and the quantitative changes of the differential counts presented in the same way confirmatory results.

#### GENERAL SUMMARY.

The study of the daily changes which take place in the blood-content of a normal person yields these main points:

The red corpuscles of the peripheral circulation do not remain constant in number per cubic millimetre, but fluctuate through a range of 5 per cent. Uniform daily changes take place in definite periods which are determined by the meal times and occupation of each individual. In the morning the number is relatively high, while near the end of the afternoon it is low. On the following morning the number of corpuscles is found again to be high.

The hæmoglobin varies directly with the number of red blood-corpuscles, and shows the same series of daily changes.

The white corpuscles, as found in the peripheral blood, vary in number per cubic millimetre only to a small extent, the range being about three thousand cells, or 20 per cent. They are lowest in the morning and highest about five o'clock in the afternoon. During the night, there occurs a steady falling off in number, until the normal mean of the morning is again reached. With the exception of the last, they increase after each meal.

The differential study shows a diminution of the per cent of polymorphonuclear leucocytes and a corresponding increase of the lymphocytes. There is an absolute increase of lymphocytes, and but little change in the number of the polymorphonuclear cells. The eosinophiles and the mast cells do not exhibit any special changes. The lymphocytes continue in absolutely increased numbers for six hours after the leucocytosis has reached its highest point.

The varieties of white corpuscles have different periods of existence in the blood, and contribute to the cellular debris of the plasma in

proportions which do not correspond to their relative percentages. The polymorphonuclear cells contribute about 49 per cent, the eosinophiles less than 1 per cent, and the lymphocytes 50 per cent.<sup>1</sup>

The nucleated red cells and myelocytes are strangers to normal blood. Small lymphocytes which possess well-defined processes, sometimes designated as "buds," amount to about 20 per cent of the class.

Inasmuch as these facts appear to have been fairly well established by additional investigations, we feel safe in saying that the blood of the healthy individual passes through a series of daily changes. The periods of the day represent a time of expenditure of energy during which there is present a fatigue curve, which rises gradually, and after which the hours of sleep represent a period of recovery.

<sup>1</sup> In this journal, 1900, iii, p. 83, MATTHEWS, in studying the origin of the fibrinogen of the blood, concludes that, "The facts (decrease of fibrinogen with extirpation of the intestines; increase of fibrinogen with artificially prolonged leucocytosis; leucocytes are going to pieces in the body; etc.) . . . point to the leucocytes of the blood, and chiefly those of the intestinal area, as being the source of the fibrinogen of the blood."

# DO THE MUCOIDS COMBINE WITH OTHER PROTEIDS?

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## I. INTRODUCTION.

THE mucoids are substances whose molecules contain glucothionic acid radicles. At present we know nothing regarding the manner in which these radicles are held in the molecule, and we understand very little about their relation to the molecular groups with which they are associated. We are justified, perhaps, in assuming that the glucothionic acid radicles in the mucoids are, in part at least, of the nature of side chains, and that these acid groups are able to combine with other radicles without severing their attachments to the molecular nucleus.

This assumption seems to be supported by the following well-known facts: Alkaline extracts of connective tissues yield, on acidification, a precipitated mass consisting chiefly of mucoid. After thoroughly washing away the acid used to precipitate it, and after its complete purification, mucoid is found to manifest marked acid properties. The moist solid substance will turn blue litmus paper red,

and it will dissolve in, and neutralize, dilute solutions of the caustic alkalies and alkaline salts.

This compound proteid does not exist in the tissues in the "free" state, but chiefly, perhaps wholly, in the form of complex neutral compounds, such, for example, as may be made when freshly precipitated mucoid (acid) is dissolved in an equimolar solution of sodium carbonate (alkaline) to a neutral, viscid, mucous fluid.<sup>1</sup>

The glucothionic acids obtainable from the mucoids have the property of combining with proteids when their salts are dissolved with the latter and the mixtures are acidified.<sup>2</sup> The products precipitated in this way are very similar to typical tissue mucoids. In all probability the various mucoids are products which differ mainly in the characters and proportions, and in the nature of the unions, of their constituent proteid and glucothionic acid radicles.

Since mucoids are acid substances because of the glucothionic acid radicles in them, and since these glucothionic acid groups unite with proteids when their salts are treated with the latter in acidified mixtures, it seems possible that mucoids themselves might unite with more proteid, if representatives of these two groups of substances were brought together in solution under favorable conditions of acidity. This statement suggests that the mucoids have varying combining powers under different conditions, that they may be similar in a way to unsaturated compounds, and that the amount of proteid with which their acid radicles or side chains may unite is variable and might be increased beyond that in the mucoids as they occur in the tissues. This research was undertaken to test these and the above deductions.

That the mucoids are variable in composition, even in the same tissue, was shown conclusively in a former research in this laboratory.<sup>3</sup> That this variability consists, in part at least, in fluctuations of the proportions of proteid and glucothionic acid radicles, was also demonstrated at the same time.

In the studies described on the following pages we have found, as

<sup>1</sup> It is possible that in diseases of the connective tissues such compounds are greatly modified or perhaps are broken up and the mucoid itself precipitated. See discussion on page 434.

<sup>2</sup> POSNER and GIES: *This journal*, 1904, xi, 341.

<sup>3</sup> CUTTER and GIES: *Proceedings of the American Physiological Society*, 1899; *This journal*, 1900, iii, p. vi; 1901, vi, p. 155. Also GIES and COLLABORATORS; *Biochemical researches*, 1903, i, Reprint No. 5.

was suspected, that, when neutral or alkaline mucoid solutions are mixed with neutral or alkaline solutions of various common proteids, and such mixed solutions are acidified, a heavy precipitate is formed. This precipitate may be several times as bulky and as heavy as the mucoid separable on acidification in control tests in the absence of the proteid referred to. In short, the glucothionic acid side chain of the mucoid appears to be able (like the glucothionic acid radicle in simple salts of the substance) to unite the mucoid with any one of various proteids to form insoluble compounds, when the two are treated, in neutral or alkaline solution, with acid in excess.

This general fact may be demonstrated very readily in the following simple manner: Treat a neutral gelatine (or other proteid) solution with a faintly acid, opalescent, mucoid solution. On the instant of contact, a bulky flocculent precipitate will be thrown down. The same or any other amount of acid alone will have no perceptible effect on the gelatine, and the neutral or alkaline fluid containing the latter will be without precipitative influence on a neutral or alkaline mucoid solution. The weight of the precipitate formed in this way exceeds that of the mucoid.

The details of our quantitative work in this connection are given below.<sup>1</sup>

#### GENERAL METHODS OF THE EXPERIMENTS.

**Mucoid.** — Tendomucoid, from the Achilles tendon of the ox, was employed in all the experiments. The methods of preparation were those of Cutter and Gies.<sup>2</sup> Some of the material was prepared just before use. Most of the samples had been obtained some time before they were needed. These were preserved in dilute alcohol (10–20 per cent) until used. The solid substance of these preparations was then freed from nearly all the alcohol by washing in water or pressing between filter papers.

**Solutions.** — Alkaline solution of mucoid was freshly prepared for each series of precipitations. The substance was dissolved in a solution of sodium carbonate, calcium hydroxide, or potassium hydroxide. The alkalinity of the solution was always relatively slight. The proteids to be associated with the mucoid were almost always dissolved in water.

<sup>1</sup> These experiments were begun by us in 1902.

<sup>2</sup> CUTTER and GIES: *Loc. cit.*



**Precipitations.** — The mucoid and secondary proteid solutions were united in varying proportions. The total volumes were the same in each series. Equal amounts of acid, usually hydrochloric acid (0.2 or 0.8 per cent), were added simultaneously to each member of the series of mixed solutions, and the precipitation-effects noted. The proper control experiments were made in each series. The precipitations were made in small beakers of equal size, so that the bulk of the precipitates could be directly compared.

**Final products.** — The precipitates obtained on acidification were eventually filtered off, thoroughly washed with water until the washings were free from soluble substance yielding the biuret reaction,<sup>1</sup> and then were dried to constant weight at 110° C. These weights are recorded in all the summaries below.

**Analyses.** — Nitrogen was determined with the Kjeldahl-Gunning method; ash, by cautious incineration in porcelain crucibles. Coagulable proteid was estimated by boiling the neutral solution and acidifying sufficiently with dilute acetic acid.

**Special methods.** — Details of special methods are given in their appropriate places in the appended summaries.

#### PRECIPITATION EXPERIMENTS.

**With gelatine.**<sup>2</sup> — I. The mucoid was freshly prepared. We used the precipitate obtained from a third limewater extract.<sup>3</sup> The substance was dissolved in a moderate excess of 0.1 per cent potassium hydroxide. An aqueous, 1 per cent solution of gelatine was employed. The essential data of this experiment are given in Table I.

II. In this experiment we used mucoid of the first extract. Half-saturated limewater was taken to dissolve it. The gelatine was in 2 per cent solution.

The plan of the experiment was the same as that indicated in Table I. The volumes differed. They were: gelatine, 80 c.c. in the control (1), 10 c.c., 40 c.c., and 80 c.c. in mixtures 3-5; mucoid, 50 c.c. in 2-5. Half-saturated limewater was used in 1 instead of water. The total volumes were 130 c.c.

<sup>1</sup> Washing was done by decantation, or the precipitates were stirred in fresh quantities of water on the funnel during the filtration process. That these procedures sufficed to remove any secondary proteid held mechanically in the precipitates was evident from the fact that the precipitates, such as albumen-mucoid, when dissolved in dilute alkali until the solution was neutral in reaction, did not become turbid or opalescent on boiling.

<sup>2</sup> FRENCH: "gold label."

<sup>3</sup> See CUTTER and GIES: *Loc. cit.*

TABLE I.  
GELATINE-MUCOID PRECIPITATES.

SOLUTIONS.	CONTROLS.		MIXTURES. <sup>1</sup>		
	Gelatine.	Mucoid.	Gelatine-mucoid.		
	1	2	3	4	5
Gelatine . . . . .	c.c. 75	c.c. ..	c.c. 10	c.c. 30	c.c. 75
Mucoid . . . . .	..	25	25	25	25
Water . . . . .	25	75	65	45	..
Total volume . .	100	100	100	100	100

INFLUENCE OF HYDROCHLORIC ACID.			
Addition. <sup>2</sup>	0.8% HCl.	Relative effects of simultaneous treatment.	
I	c.c. 2	Clear	Increasing turbidity from 2 to 5.
II	2	Clear	Milky
III	2	Clear	Milky
IV	2	Clear <sup>5</sup>	Precipitate <sup>6</sup>
			Increasing precipitates from 3 to 5. <sup>3</sup> Further increase in precipitates 3 to 5. <sup>4</sup> No further increase in precipitates 3 to 5. <sup>7</sup>

WEIGHTS OF PRECIPITATES. <sup>8</sup>					
	gram 0.0	gram 0.3701	gram 0.4180	gram 0.4707	gram 0.6348

<sup>1</sup> All of the mixtures were clear.

<sup>2</sup> The additions were made at 3.30, 3.35, 4.10, and 4.15 P. M.

<sup>3</sup> The precipitates were fairly heavy; precipitation was incomplete.

<sup>4</sup> The precipitate in 5 settled through a clear fluid; the supernatant fluid in each of 3 and 4 was milky. The milkiness was more decided in 3 than in 4. The precipitates were somewhat gelatinous.

<sup>5</sup> Acid had no perceptible influence.

<sup>6</sup> On standing over night the supernatant fluid became clear.

<sup>7</sup> Precipitates 3-5 resembled aluminium hydroxide.

<sup>8</sup> All of the precipitates were left standing over night. They showed a staircase effect, rising from 2 to 5.

The acid, 0.8 per cent hydrochloric, was added, from 2 : 30–5 : 35 P.M., in quantities of 2 c.c. The results were practically the same as before, except that 10 c.c. of the acid were necessary to develop them fully. This increase was required by the larger total volumes. The gelatinous effects in 4 and 5 were even more marked than before. Portions of the precipitates in 4 and 5 had redissolved, or become more finely divided, and it was impossible to filter these mixtures.

The following weights were taken :

Controls.		Gelatine-Mucoid Precipitate.
1. Gelatine.	2. Mucoid.	3.
0.0	0.0973 gm.	0.1651 gm.

III. Mixed mucoid was used in this experiment. It was dissolved in 0.5 per cent sodium carbonate. A fairly dilute gelatine solution was used. The plan of the experiment was the same as that indicated in Table I. The volumes were also the same. The acid taken for the precipitations was 0.2 per cent hydrochloric, which was added in volumes of 5 c.c.

We obtained essentially the same effects as those recorded in Table I, although greater volumes of acid had to be employed to show maximum results. The first flocculent precipitate was obtained in 3. The precipitates in 4 and 5 were too gelatinous for gravimetric treatment in the time at our disposal. The precipitates from 2–5 showed a staircase effect (rising from 2 to 5), which was even more marked than in the first two experiments.

The weights obtained were as follows :

Controls.		Gelatine-Mucoid Precipitate.
1. Gelatine.	2. Mucoid.	3.
0.0	0.1442 gm.	0.1602 gm.

IV. All conditions of the last experiment were duplicated except the strength of the mucoid and gelatine solutions. A dilute solution of the former was used; the latter was a 2.5 per cent solution. The results were qualitatively about the same as before. Precipitate 5 was again too gelatinous for successful gravimetric treatment. The following quantitative data were obtained :

Controls.		Gelatine-Mucoid Precipitates.	
1. Gelatine.	2. Mucoid.	3.	4.
0.0	0.1462 gm.	0.1676 gm.	0.2096 gm.

V. The first experiment was duplicated in all respects until the precipitate in 5 seemed to be complete. The usual qualitative results

were noted. When 5 was completely precipitated, 2, the mucoid control, was still milky and had not become flocculent; 3 and 4 seemed to be completely flocculent. In order to compare precipitates 3-5 with the mucoid in each, 2 was further treated with 4 c.c. of 0.8 per cent hydrochloric acid to effect its complete precipitation. The following gravimetric data were obtained:

Controls.		Gelatine-Mucoid Precipitates.		
1. Gelatine.	2. Mucoid.	3.	4.	5.
0.0	0.2825 gm.	0.3419 gm.	0.4751 gm.	0.6863 gm.

VI. Experiment V was repeated, but the additions were carried forward simultaneously until 2 had been completely precipitated. The results noted in the first experiment were duplicated here, except that the precipitate in 3 was much less gelatinous in appearance than before. The weights are given below:

Controls.		Gelatine-Mucoid Precipitates.		
1. Gelatine.	2. Mucoid.	3.	4.	5.
0.0	0.2626 gm.	0.3306 gm.	0.4401 gm.	0.6167 gm.

VII. All the conditions of the first experiment were duplicated except the strength of the gelatine solution, which, in this case, was only 0.1 per cent. Only the initial effect of acid was determined. On adding 2 c.c. of 0.8 per cent hydrochloric acid to each of the five mixtures, the following results were noted:

1.	2.	3.	4.	5.
Gelatine Control.	Mucoid Control.	Gelatine-Mucoid.	Gelatine-Mucoid.	Gelatine-Mucoid.
No effect.	Opalescence.	Deep milkiness.	Slight precipitate.	Heavy precipitate.

Additional results with gelatine are indicated in Experiment XXXI. In that experiment the nitrogen content of the various precipitates was determined.

With proteoses (Witte's "peptone"). — VIII. Mixed mucoid was taken. It was dissolved in 0.5 per cent sodium carbonate solution. Witte's "peptone" was dissolved in water and the clear filtrate taken. The essential data of this experiment are given in Table II.

IX. The eighth experiment was repeated. All conditions were essentially the same, except that the proteose solution (3 per cent) in water was neutralized before it was filtered.

The precipitative effects were much the same. On adding the first 5 c.c. of 0.2 per cent hydrochloric acid, all of the mixtures became

TABLE II (FIRST PART).  
PROTEOSE-MUCOID PRECIPITATES.

SOLUTIONS.	CONTROLS.		MIXTURES. <sup>1</sup>		
	Witte's "peptone."	Mucoid.	Witte's "peptone"-mucoid.		
	1	2	3	4	5
Witte's "peptone" . . .	c.c. 75	c.c. ..	c.c. 10	c.c. 30	c.c. 75
Mucoid . . . . .	..	25	25	25	25
Water . . . . .	25	75	65	45	..
Total volume . . . .	100	100	100	100	100

INFLUENCE OF HYDROCHLORIC ACID.				
Addition. <sup>2</sup>	0.2% HCl.	Relative effects of simultaneous treatment.		
I	c.c. 25 <sup>3</sup>	Slight precipitate <sup>4</sup>	Milky	Increasing precipitates from 3 to 5. <sup>5</sup>
II	5	Unchanged	Slight precipitate	Further increase in precipitates 3 to 5. <sup>6</sup>
III	25 <sup>7</sup>	Unchanged	Heavy precipitate <sup>8</sup>	Further increase in precipitates 4 and 5. <sup>9</sup>
IV	15 <sup>10</sup>	Unchanged	Unchanged	Further increase in precipitate 5. <sup>11</sup>
V	30 <sup>12</sup>	Unchanged	Unchanged	No further increase in any except 5. <sup>13</sup>

<sup>1</sup> 1, 3, 4, and 5 were turbid. The turbidity was more decided in 1, than in any other.

<sup>2</sup> All additions were made in 5 c.c. volumes at intervals of at least five minutes.

<sup>3</sup> The additions were made at intervals of five minutes, from 4.55 to 5.15 P.M.

<sup>4</sup> The proteose solution had not been previously neutralized.

<sup>5</sup> The first precipitation of the series occurred in 5.

<sup>6</sup> The supernatant fluids showed increasing milkiness from 3 to 5.

<sup>7</sup> One volume had been added at 9.10 P.M., the remaining portions at 3.45-4.00 P.M. on the following day.

<sup>8</sup> Precipitation was complete after the addition of 10 c.c.

<sup>9</sup> The usual staircase effect was observed.

<sup>10</sup> Added at 6 to 6.15 P. M.

<sup>11</sup> No perceptible effects on 3 and 4.

<sup>12</sup> One half added at 10 A. M. on the third day, the other half at 10 A. M. on the fourth day.

<sup>13</sup> Precipitation in 5 was complete after the addition of 20 c.c.

TABLE II (SECOND PART).

WEIGHTS OF PRECIPITATES. <sup>1</sup>					
	CONTROLS.		MIXTURES.		
	Witte's "peptone."	Mucoid.	Witte's "peptone"-mucoid.		
	1	2	3	4	5
Weights obtained . . . .	gram 0.0513	gram <b>0.1487</b>	gram 0.1577	gram 0.1836	gram .. <sup>2</sup>
Proteose as per control . .	0.0513	..	0.0068	0.0204	..
Proteose-mucoid . . . .	..	..	<b>0.1509</b>	<b>0.1632</b>	..
<sup>1</sup> The staircase effect was inconspicuous 24 hours after precipitation. <sup>2</sup> Precipitate 5 could not be thoroughly washed.					

turbid. Later additions reduced the turbidity in 1, and gave flocculent precipitates in 3-5 before flakes appeared in 2. Acid was added until precipitation in 2 was complete,<sup>1</sup> with results like those previously recorded. The following quantitative data were obtained :

Controls.		Proteose-Mucoid Precipitates. <sup>2</sup>		
1. Proteose.	2. Mucoid.	3.	4.	5.
0.0027 gm.	0.0517 gm.	0.1004 gm.	0.1165 gm.	0.1494 gm.

X. Experiment VIII was again repeated. Former conditions were duplicated, except that mucoid of a fourth extract was dissolved in 0.1 per cent potassium hydroxide, and all of the proteose was combined with hydrochloric acid before it was used in the mixtures. 350 c.c. of a 3 per cent aqueous mixture of Witte's "peptone" were treated with 0.8 per cent hydrochloric acid until only a trace of free acid could be detected with tropæolin oo. The clear filtrate was used to make up mixtures similar to those indicated in Table II.

On making up 5 a flocculent precipitate was formed, due, evidently, to the effect of the combined acid in the proteose solution.

The precipitative effects on adding acid were relatively the same as before, except that the proteose control, (1), failed to yield a precipitate.

<sup>1</sup> In this experiment 90 c.c. sufficed. In the former proteid 100 c.c. were taken.

<sup>2</sup> Corrections, on the basis of the result in the proteose control, were made on 3, 4, and 5 for the quantity of proteose precipitated in them by the acid.

At first, 3, 4, and 5 were completely precipitated, while 2 remained milky. Acidification was carried forward to complete precipitation in 2-5, with the following gravimetric results:

Controls.		Proteose-Mucoid Precipitates.		
1. Proteose.	2. Mucoid.	3.	4.	5.
0.0	0.4110 gm.	0.4260 gm.	0.4386 gm.	0.4571 gm.

**With muscle proteids.**—XI. The mucoid was obtained from a fourth tendon extract, and was dissolved in 0.5 per cent potassium hydroxide. A concentrated aqueous extract of fresh beef was used. The essential facts of this experiment may be noted in Table III.

XII. Experiment XI was repeated. The aqueous extract of the hashed meat was made at 40° C. The mucoid was obtained from a second tendon extract. Otherwise all conditions were identical. Essentially the same results were noted as before. Precipitation occurred in the same order. Precipitate 2 was snow-white, 5 was brown, 3 and 4 were intermediate in color. All the mucoid filtrates except 5 were colorless.<sup>1</sup> Filtrate 5 retained a slight reddish tinge. On washing the precipitates, the color was not discharged. 6 c.c. of the acid sufficed to precipitate completely the mucoid in 2. An additional volume (2 c.c.) was added to each beaker to produce moderately excessive acidity. The following weights were obtained:

Controls.		Muscle Proteid-Mucoid Precipitates.		
1. Meat Extract.	2. Mucoid.	3.	4.	5.
0.0 <sup>2</sup>	0.4648 gm.	0.6272 gm.	0.8819 gm.	1.1419 gm.

XIII. Experiment XI was again repeated. All conditions were the same, except that the mucoid was obtained from the first tendon extract. 12 c.c. of the acid gave complete precipitation of the mucoid control. An excess of 25 c.c. of 0.8 per cent hydrochloric acid was added to each beaker, and the mixtures allowed to stand. The

<sup>1</sup> No such decolorization is effected when a mucoid *precipitate* is transferred to a watery extract of meat and thoroughly stirred in the latter. Our results in this connection appear to be due to a direct union between the mucoid and the proteid coloring matter.

<sup>2</sup> The slight precipitate in the meat-extract control dissolved on washing as it did in the previous experiment. In this experiment we made no attempt to recover it.

TABLE III.  
MUSCLE PROTEID-MUCOID PRECIPITATES.

		CONTROLS.		MIXTURES. <sup>1</sup>		
SOLUTIONS.		Muscle extract.	Mucoid.	Muscle proteid-mucoid.		
		1	2	3	4	5
	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.
Muscle extract . .	75	..	10	30	75	
Mucoid . . . . .	..	25	25	25	25	
Water . . . . .	25	75	65	45	..	
Total volume .	100	100	100	100	100	100

INFLUENCE OF HYDROCHLORIC ACID.				
Addition. <sup>2</sup> 0.8% HCl.		Relative effects of simultaneous treatment.		
I	c.c. 2	Precipitate <sup>3</sup>	No effect, 2-5	
II	2	Precipitate diminishing <sup>4</sup>	No effect, 2-4	Turbid.
III	2	Precipitate almost gone	Milky	Increasing precipitates, 3-5. <sup>5</sup>
IV	2	Turbid	Precipitate	Further increase in precipitates, 3-5. <sup>6</sup>
V	2	Less turbid	No change, 2-5. <sup>7</sup>	

WEIGHTS OF PRECIPITATES. <sup>8</sup>					
	gram 0.0257 <sup>9</sup>	gram 0.4057	gram 0.4983 <sup>10</sup>	gram 0.7119	gram 1.0399

<sup>1</sup> All of the mixtures were clear. 3, 4, and 5 were bloody.

<sup>2</sup> The additions were made at varying intervals on the same afternoon.

<sup>3</sup> Globulin (?).

<sup>4</sup> As the precipitate diminished, the solution took on its original appearance.

<sup>5</sup> Under reddish milky fluids.

<sup>6</sup> The precipitate settled out rapidly under *colorless water-clear fluids*. The hæmoglobin in the original solutions was held by the precipitates. See footnote 1, page 413.

<sup>7</sup> The excess of acid failed to dissolve the precipitates, whereas in the control (1), further solution occurred.

<sup>8</sup> The precipitates showed a striking staircase effect, rising rapidly from 2 to 5.

<sup>9</sup> The slight precipitate in 2 *dissolved* on washing it with water, but the washings gave a precipitate on mixing with the original filtrate. This precipitate was collected and dried without washing. Its weight is given as that for the muscle-extract control, but no deductions are made on its account from the figures for precipitates 2-5.

<sup>10</sup> Precipitates 3-5 retained their bloody color throughout the entire washing process.



various filtrates were colorless, and the usual staircase appearance of the precipitates was noted. The following data were obtained :

	Controls.		Muscle Proteid-Mucoid Precipitates.		
	1. Meat Extract.	2. Mucoid.	3.	4.	5.
<i>Dry substance</i>	0.0045 gm. <sup>1</sup>	0.5181 gm.	0.5726 gm.	0.6717 gm.	0.8138 gm.
<i>Ash</i> . . .	0.0009 "	0.0013 "	0.0014 "	0.0068 "	0.0141 "

XIV. In this experiment muscle alkali albuminate was used. Fresh beef was extracted over night in 0.1 per cent potassium hydroxide. The filtrate was neutralized with 0.1 per cent hydrochloric acid. The solid albuminate was redissolved in 0.1 per cent potassium hydroxide. Mixed mucoid was also dissolved in that strength of caustic potash. All other conditions were the same as in Experiment XI, except that 40 c.c. of the albuminate solution, instead of 30, were used in 4, with the corresponding change in the complementary volume of water.

On adding 2 c.c. of 0.8 per cent hydrochloric acid, a flocculent precipitate formed in 1, there were turbidities in 2 and 3, no effect in 4 and 5. A second addition of 2 c.c. of the acid caused complete solution of precipitate 1, and heavy flocculent precipitations in 2-5. A further addition of 2 c.c. of the acid seemed to be without effect. On standing over night, the precipitates showed the usual staircase effect. The following weights were obtained :

	Controls.		Albuminate-Mucoid Precipitates.		
	1. Albuminate.	2. Mucoid.	3.	4.	5.
	0.0	0.1794 gm.	0.2310 gm.	0.3086 gm.	0.4305 gm.

XV. Muscle acidalbumin was used in this experiment. It was made with 0.1 per cent hydrochloric acid, and precipitated with 0.1 per cent potassium hydroxide. It was redissolved in the latter solution. All other conditions were the same as those in the preceding experiment. The precipitative results were essentially the same. The acidalbumin precipitates, however, were somewhat gelatinous and viscid, and so difficult to wash and filter that only the following weights could be obtained :

	Controls.		Acidalbumin-Mucoid Precipitate.	
	1. Acidalbumin.	2. Mucoid.	3.	
	0.0	0.1883 gm.	0.2375 gm.	

Experiment XXXII gives further qualitative and quantitative data in connection with muscle proteïds.

<sup>1</sup> This precipitate was dried without washing. See footnote 2, page 413.

**With tendon proteids.**—XVI. Experiment XI was repeated in all respects, except that a concentrated aqueous tendon extract was taken and the mucoid, from a third extract of tendon, was dissolved in 0.1 per cent potassium hydroxide. The tendon extract contained only minute traces of hæmoglobin. Essentially the same precipitative effects were seen as those recorded in Table III. The following weights were obtained :

Controls.		Tendon Extract-Mucoid Precipitates.		
1. Tendon Extract. <sup>1</sup>	2. Mucoid.	3.	4.	5.
0.0	0.2596 gm.	0.2948 gm.	0.3245 gm.	0.3189 gm. <sup>2</sup>

**With serum proteids.**—XVII. The mucoid had been prepared from a second tendon extract, and was dissolved in 0.1 per cent potassium hydroxide. Ox serum, which had been preserved in a dry condition, was dissolved in an 0.5 per cent solution of sodium chloride. The more important facts of the experiment are given in Table IV.

XVIII. Experiment XVII was repeated with the following changes : The serum was dissolved in water. Mucoid of a first extract was taken and dissolved in half-saturated limewater. The solution was much more dilute than in Experiment XVII. 50 c.c. of mucoid solution were taken in 2 to 5. The serum volumes in 3, 4, and 5 and 1 were 10 c.c., 40 c.c., and 80 c.c. The total volume in each was 130 c.c. Water, instead of 0.5 per cent sodium chloride, was used as the complementary fluid, but in 1, 50 c.c. of half-saturated limewater were added to the serum to make up the total volume. This mixture stood for a half hour before acid treatment was begun.

On adding 2 c.c. of 0.8 per cent hydrochloric acid, 1 remained unchanged, 2 was opalescent, 3 milky, and 4 and 5 were precipitated. 1 was alkaline,<sup>3</sup> 2, 4, and 5 neutral, and 3 acid. After again adding 2 c.c. of the acid, 1 became flocculent, 2 milky, 3 flocculent, 4 more flocculent, 5 less flocculent. All were acid, though in different degree. Further additions of acid were made until the volume was increased by 6 c.c.<sup>4</sup> This amount was required to precipitate 2. On standing over night, 1 was entirely clear, 2 was completely precipitated, 3 collected under a clear fluid, 4 settled under a slightly

<sup>1</sup> We have repeatedly observed that aqueous extracts of tendon fail to yield mucoid on acidification. On some occasions, however, a slight precipitate may be obtained under these conditions. See page 434.

<sup>2</sup> This result seems to have been affected by some unobserved error.

<sup>3</sup> To litmus.

<sup>4</sup> The additions of acid were made in 2 c.c. volumes at intervals of a half hour.

TABLE IV.  
SERUM PROTEID-MUCOID PRECIPITATES.

SOLUTIONS.	CONTROLS.		MIXTURES. <sup>1</sup>		
	Serum.	Mucoid.	Serum-mucoid.		
	1	2	3	4	5
Serum . . . . .	c.c. <b>75</b>	c.c. ..	c.c. <b>10</b>	c.c. <b>30</b>	c.c. <b>75</b>
Mucoid . . . . .	..	25 <sup>2</sup>	25	25	25
0.5% NaCl . . . . .	25	75	65	45	..
Total volume . .	100	100	100	100	100

INFLUENCE OF HYDROCHLORIC ACID.				
Addition. <sup>3</sup>	0.8% HCl.	Relative effects of simultaneous treatment.		
I	c.c. 8	Clear	Opalescent	Increasing milkiness from 3 to 5.
II	2	Clear	Milky	Increasing precipitates from 3 to 5. <sup>4</sup>
III	2	Clear	Precipitate	No change, 3-5. <sup>5</sup>
IV	2	Clear	No change, 2-5. <sup>6</sup>	

WEIGHTS OF PRECIPITATES. <sup>7</sup>					
	gram 0.0147	gram 0.3224	gram 0.5295	gram 0.7598	gram 1.2306

<sup>1</sup> The mixture in 2 was slightly turbid.

<sup>2</sup> On acidifying, the saline content of the mucoid control was approximately equal to that in 1.

<sup>3</sup> All of the additions were made on the same afternoon at intervals of from 5 to 10 minutes.

<sup>4</sup> Precipitate 5 settled more rapidly than the others. Each seemed to be completely separated.

<sup>5</sup> No solution was apparent.

<sup>6</sup> The excess of acid appeared to be without solvent effect, for the supernatant fluids were water-clear.

<sup>7</sup> After standing all night, the usual staircase effect was seen.

turbid solution, 5 was viscid, with no sediment. The latter could not be filtered and properly washed, but the following weights were obtained :

Controls.		Serum-Mucoid Precipitates.	
1. Serum.	2. Mucoid.	3.	4.
0.0	0.0955 gm.	0.1351 gm.	0.1529 gm.

XIX. Experiment XVIII was repeated with dog serum in stronger solution (5 per cent). Mixed mucoid was prepared from a fresh lot of tendons, after the latter had been washed in running water for twenty-four hours. Water was used in the serum control (1), instead of limewater, to make up the total volume. Essentially the same precipitative results were obtained, except that 3 and 4 were the viscid precipitates in this case, 5 was more flocculent.<sup>1</sup> We obtained the following figures for weights of the precipitates :

Controls.		Serum-Mucoid Precipitates.		
1. Serum.	2. Mucoid.	3.	4.	5.
0.0	0.0915 gm.	—	—	0.2891 gm.

**With egg albumen.**—XX. A concentrated solution of mixed mucoid was made in 0.5 per cent potassium hydroxide. The albumen from two eggs was thoroughly shaken in 250 c.c. of water, and the filtrate used in the mixtures indicated in Table V, where the essential data of this experiment are recorded.

XXI. Experiment XX was repeated under the following changed conditions : The mucoid, from the first tendon extract, was dissolved in half-saturated limewater. The volume of mucoid solution in each beaker (2-5) was 50 c.c. The volumes of albumen solution were 10 c.c. in 3, 40 c.c. in 4, and 80 c.c. in 5 and 1. 50 c.c. of half-saturated limewater were used as the supplemental volume in the albumen control. The total volume was 130 c.c. in each beaker. Essentially the same precipitative effects were obtained as before, except that after the addition of 6 c.c. of the acid,<sup>2</sup> flocculent precipitates were obtained in 3, 4, and 5, whereas 2 was merely milky. Precipitation occurred first in 3. A total of 12 c.c. of 0.8 per cent hydrochloric acid was added—a moderate excess. After standing over night, 2, 3, 4, and 5 were completely precipitated, 1 was clear.

<sup>1</sup> A total of 12 c.c. of 0.8 per cent hydrochloric acid was added to each.

<sup>2</sup> This amount was sufficient to acidify all the mixtures.

TABLE V.  
EGG ALBUMEN-MUCOID PRECIPITATES.

SOLUTIONS.	CONTROLS.		MIXTURES. <sup>1</sup>		
	Egg albumen.	Mucoid.	Egg albumen-mucoid.		
	1	2	3	4	5
Egg albumen . . . . .	c.c. 75	c.c. ..	c.c. 10	c.c. 30	c.c. 75
Mucoid . . . . .	..	25	25	25	25
Water . . . . .	..	75	65	45	..
0.5% KOH . . . . .	25	..	..	..	..
Total volume . . . .	100	100	100	100	100
INFLUENCE OF HYDROCHLORIC ACID					
Addition. <sup>2</sup>	0.8% HCl.	Relative effects of simultaneous treatment.			
I	c.c. 6	Unchanged	Increasing milkiness from 2 to 5.		
II	2	Unchanged	Increasing precipitates from 2 to 5.		
III	2	Unchanged	Large flocks in 2-4; small in 5. <sup>3</sup>		
IV	2	Flocculent <sup>4</sup>	Large flocks in 2-5. <sup>5</sup>		
V	2	Same	Precipitates in 4 and 5 increasing. <sup>6</sup>		
VI	2	Same	No further change. <sup>7</sup>		
WEIGHTS OF PRECIPITATES. <sup>8</sup>					
	gram 0.0433 <sup>9</sup>	gram 0.6807	gram 0.7766	gram 0.8204	gram 1.0327
<sup>1</sup> All were clear except 1, which was moderately turbid. <sup>2</sup> All additions were made during the same afternoon, at intervals of about a quarter of an hour. <sup>3</sup> Precipitation was less advanced in 5. <sup>4</sup> The particles causing turbidity in the first place now came together, but there was no apparent increase in the amount of the floating substance. <sup>5</sup> The supernatant fluids in 4 and 5 were still milky. <sup>6</sup> All supernatant fluids were now water-clear. <sup>7</sup> The excess of acid had no apparent solvent action. <sup>8</sup> Striking staircase effect, rising from 2 to 5, was observed even after 24 hours' standing. <sup>9</sup> No corrections were made on account of this slight precipitate.					

The usual staircase appearance of the precipitates was seen. Figures or the weights of the precipitates follow :

Controls.		Egg Albumen-Mucoid Precipitates.		
1. Egg Albumen.	2. Mucoid.	3.	4.	5.
0.0	0.0953 gm.	0.1300 gm.	0.1592 gm.	0.2225 gm.

XXII. Experiment XX was repeated with the following variations: Mixed mucoid was dissolved in 0.5 per cent sodium carbonate. Commercial dry albumen was dissolved in water, and the clear filtrate employed. Water was used throughout to make up the total volume to 100 c.c. 30 c.c. of mucoid solution were used in 2, 3, and 4. [5 was omitted.] The volume of albumen solution was 30 c.c. in 3 and 60 c.c. in 4 and 1. 0.2 per cent hydrochloric acid was used to precipitate.

After adding 10 c.c. of the acid,<sup>1</sup> 1 remained clear, 2 became opalescent, 3 was partly precipitated, and 4 seemed to be wholly precipitated. After adding 10 c.c. more of acid, precipitates 3 and 4 were bulky, and both seemed to be complete; 2 was still only milky, 1 unchanged. It required a further addition of 40 c.c. of the acid<sup>2</sup> to completely precipitate the mucoid in the control (2). This excess of acid seemed to be without solvent influence on precipitates 3 and 4, and without effect on 1. After standing over night, all precipitates settled under water-clear solutions, and the albumen control was without sediment. The precipitates showed the usual staircase effect. The following weights were obtained :

Controls.		Egg Albumen-Mucoid Precipitates.	
1. Egg Albumen.	2 Mucoid.	3.	4.
0.0	0.2317 gm.	0.4237 gm.	0.5761 gm.

XXIII. Experiment XX was again repeated under somewhat changed conditions, as follows: 52 grams of moist mucoid, of a second extract, were dissolved in 400 c.c. of 0.5 potassium hydroxide. Dilute sulphuric acid (1-10) was used to precipitate. 65 c.c. of the mucoid solution were used in 2-7. The volumes of albumen solution were 5 c.c., 10 c.c., 20 c.c., 40 c.c., in 3, 4, 5, and 6 respectively, and 80 c.c. in 7 and in 1. Water was used to make up the total volumes to 200 c.c.<sup>3</sup> The acid was added in 2 c.c. portions.

<sup>1</sup> The acid was added in 5 c.c. volumes at varying intervals.

<sup>2</sup> Added in 5 c.c. volumes at intervals of about an hour on the following day.

<sup>3</sup> After precipitation had been completed, 40 c.c. of water were added to make the total volume 250 c.c. in each case.

5 c.c. of the acid sufficed to completely precipitate. 3, 4, 5, 6, and 7 came down before 2.<sup>1</sup> The general effects noted in experiments XX-XXIII were duplicated. The staircase appearance of the precipitates was as marked as usual. The following weights were obtained:

Controls.		Egg Albumen-Mucoid Precipitates.				
1. Egg Albumen.	2. Mucoid.	3.	4.	5.	6.	7.
0.0	2.204 gms. <sup>2</sup>	2.431 gms.	2.565 gms.	2.751 gms.	3.280 gms. <sup>3</sup>	4.023 gms. <sup>4</sup>

XXIV. Experiment XX was exactly duplicated. The same precipitative effects were observed. On standing over night, the precipitates were in the same staircase relation repeatedly noted before. No precipitate formed in 1. No weights were taken.

XXV. Experiment XX was repeated with the following altered conditions: Egg albumen was dissolved in water in the proportion of 1 in 10. The mucoid was taken from a second extract. 2 per cent acetic acid was taken to effect precipitation. 40 c.c. of albumen solution were used in 4.

After adding 4 c.c. of the acid, 3, 4, and 5 were precipitated, 2 was merely milky and 1 and 5 were unchanged. After adding acid to complete precipitation of 2,<sup>5</sup> and allowing to stand over night, all precipitates had collected under relatively clear solutions, and the usual staircase positions had been assumed. The following weights were recorded:

Controls.		Egg Albumen-Mucoid Precipitates.		
1. Egg Albumen.	2. Mucoid.	3.	4.	5.
0.0	0.1336 gm.	0.2039 gm.	0.2886 gm.	0.3498 gm.

XXVI. Experiment XXV was repeated with an 0.5 per cent sodium carbonate solution of mixed mucoid. The precipitative effects were practically identical, as was also the staircase appearance of the precipitates on standing.

XXVII. In this experiment we ascertained some facts regarding the filtrates from the egg albumen-mucoid precipitates. 15 grams of moist mucoid from a second extract were dissolved in 210 c.c. of half-

<sup>1</sup> Precipitation occurred first in the beakers containing the least amount of albumen solution.

<sup>2</sup> The nitrogen content was 11.95 per cent. See Table X.

<sup>3</sup> The nitrogen content was 12.60 per cent. See Table X.

<sup>4</sup> The nitrogen content was 13.04 per cent. See Table X.

<sup>5</sup> 10 c.c. in addition were required.

saturated limewater. The whites of two eggs were dissolved in 250 c.c. of water. Mixtures were made as indicated in Table VI. 0.8 per cent hydrochloric acid was used to precipitate. The usual precipitative effects were observed. Aliquot portions of the filtrates were carefully neutralized; and the effects noted. The coagulable proteid in the neutral fluids was determined gravimetrically by the customary process. Further data will be found in Table VI.

XXVIII. Does mucoid precipitate the associated proteid by entering into chemical combination with it under the conditions of these experiments, or is it merely carried down mechanically by the mucoid? We tried to answer this question more definitely in the following two experiments:

Mucoid of a third extract was dissolved in 0.1 per cent potassium hydroxide. Egg albumen was dissolved in water in the proportion of 1 of the former to 4 of the latter.

The following mixtures were made up and treated as indicated below:

1. *Albumen control*. — 50 c.c. of the albumen solution + 50 c.c. of water + 8 c.c. of 0.8 per cent hydrochloric acid.<sup>1</sup> No precipitative effect. The mixture was allowed to stand until the following day, with the same negative result.

2. *Mucoid control*. — 50 c.c. of the mucoid solution + 50 c.c. of water. 8 c.c. of 0.8 per cent hydrochloric acid completely precipitated the mucoid.

3. *Washed mucoid in albumen solution*. — 50 c.c. of the mucoid solution + 50 c.c. of water. After complete precipitation with 8 c.c. of 0.8 per cent hydrochloric acid, the filtrate was used as solution 4 (below), and the precipitate was thoroughly washed free from adherent acid. The precipitate was then transferred to 50 c.c. of water, to which 50 c.c. of the albumen solution was added. The mixture was thoroughly stirred<sup>2</sup> and allowed to stand for the usual length of time.

4. *Acid filtrate from precipitated mucoid, mixed with albumen solution*. — 25 c.c. of the acid filtrate from 3 were treated with 25 c.c. of the albumen solution, and the two fluids thoroughly mixed. Only a very delicate turbidity ensued. The mixture was allowed to stand

<sup>1</sup> This quantity was sufficient to completely precipitate the mucoid products in the remaining mixtures.

<sup>2</sup> The precipitate was just as finely flocculent at this point as when freshly precipitated.



TABLE VI (FIRST PART).

EGG-ALBUMEN-MUCOID PRECIPITATES AND FILTRATES.

SOLUTIONS.	CONTROLS.		MIXTURES. <sup>1</sup>		
	Egg albumen.	Mucoid.	Egg albumen-mucoid.		
	1	2	3	4	5
Egg albumen . . . . .	c.c. 80	c.c. ..	c.c. 10	c.c. 40	c.c. 80
Mucoid . . . . .	..	50	50	50	50
Water . . . . .	..	80	70	40	..
Half-saturated lime-water . . . . .	50	..	..	..	..
Total volume . . . .	130	130	130	130	130

INFLUENCE OF HYDROCHLORIC ACID.						
Addition. <sup>2</sup>	0.8% HCl.	Relative effects of simultaneous treatment.				
I	c.c. 2	Less turbid	Turbid	Milky	Milky	Turbid.
II	2	Less turbid	Flocculent	Flocculent <sup>3</sup>	Flocculent	Milky. <sup>4</sup>
III	2	Less turbid	Completely precipitated	Increased precipitate		Flocculent. <sup>5</sup>
IV	2	Clearing	Completely precipitated			Increased precipitate.
V	2	Clear	Completely precipitated			
VI	2	No change, 1-5. <sup>6</sup>				

<sup>1</sup> All were clear at the start except 1, which was decidedly turbid.

<sup>2</sup> Additions were made at intervals of 5 and 10 minutes.

<sup>3</sup> Precipitate 3 seemed to be more complete than either 2 or 4.

<sup>4</sup> The mixture was still slightly alkaline.

<sup>5</sup> Faintly acid; 2-4 decidedly acid; 1 faintly acid.

<sup>6</sup> The excess of acid appeared to be without solvent action. The precipitates, on standing, exhibited the usual staircase effect.

TABLE VI (SECOND PART).

GRAVIMETRIC DATA.					
	CONTROLS.		MIXTURES.		
	Egg albumen.	Mucoid.	Egg albumen-mucoid.		
	1	2	3	4	5
	gram	gram	gram	gram	gram
Weights of egg albumen-mucoid precipitates . . . . .	0.0	0.6251	0.8687	1.1780	1.4980
Coagulable proteid in filtrates <sup>1</sup> . . . . .	1.6127	0.0060	0.0066	0.2971	0.6376
Coagulable proteid combined in the albumen-mucoid precipitates (calculated) <sup>2</sup> . .	..	..	0.2436	0.5529	0.8729
Total coagulable proteid accounted for . .	1.6127	..	0.2502	0.8500	1.5105
Total coagulable proteid in each original mixture <sup>3</sup> . . . . .	1.6127	0.0000	0.2016	0.8063	1.6127
Difference <sup>4</sup> . . . . .	..	..	+0.0486	+0.0437	-0.1022

<sup>1</sup> On neutralizing, all of the filtrates were clear except 1, which returned to its original turbidity.

<sup>2</sup> It was assumed that each of 3-5 contained its relative proportion of 0.6251 gram of mucoid, the quantity in 2.

<sup>3</sup> It was assumed that each of 3-5 contained its relative proportion of 1.6127 gram of coagulable proteid, the quantity in 1.

<sup>4</sup> Slight, in spite of the mechanical difficulties at every turn.

over night with the others. The quantity of precipitate did not increase in the meantime.<sup>1</sup>

5. *Mucoid, in ACID fluid, plus albumen solution.*—50 c.c. of the mucoid solution + 8 c.c. of 0.8 per cent hydrochloric acid to complete precipitation. 50 c.c. of the albumen solution were added to the flocculent acid mixture,<sup>2</sup> and the whole was thoroughly stirred and allowed to stand over night.

<sup>1</sup> Further additions of acid were also without effect.

<sup>2</sup> This addition was seen to result in immediate increase of the precipitate.

6. *Mucoid and albumen mixed in ALKALINE solution and together treated with acid.*—50 c.c. of the mucoid solution + 50 c.c. of the albumen solution + 8 c.c. of 0.8 per cent hydrochloric acid. The mixture was thoroughly stirred and put away over night with the preceding mixtures.

The gravimetric data of this experiment are given in Table VII.

XXIX. Experiment XXVIII was repeated. Mucoid of a second extract was dissolved in half-saturated limewater. A 1 to 15 aqueous solution of white of egg was used. 8 c.c. of 0.8 per cent hydrochloric acid sufficed for complete separation of the precipitable proteids in the mixtures, which were made up as before. Our results are summarized in Table VII. Experiment XXXIII gives additional data—precipitative effects as well as nitrogen contents, etc., of the compounds.

TABLE VII.

EGG ALBUMEN-MUCOID PRECIPITATES, ETC. EXPERIMENTS XXVIII AND XXIX.

EXPERIMENT.	CONTROLS.		EGG ALBUMEN-MUCOID MIXTURES.			
	Egg albumen.	Mucoid.	Washed mucoid (solid) in albumen solution; acid absent.	Acid mucoid filtrate mixed with albumen solution.	Precipitated mucoid in its acid solution plus albumen solution.	Mixed albumen and mucoid solutions plus acid.
No.	1	2 <sup>1</sup>	3	4	5	6
XXVIII	gram 0.0	gram 0.3746	gram 0.3233	gram 0.0	gram 0.7883	gram 0.7990
XXIX	0.0	0.1669	0.1793	0.0	0.4180	0.4019

<sup>1</sup> In each experiment the quantity of mucoid was exactly the same in 2, 3, 5, and 6. Compare 2 and 3, 5 and 6, and the first of these pairs with the second.

**With glycogen.**—XXX. Mucoid of a second extract was dissolved in half-saturated limewater. Glycogen, made from scallops, was dissolved in water and a 4 per cent solution used. The following mixtures were made up:

1. *Glycogen control.* 75 c.c. of the glycogen solution + 25 c.c. of half-saturated limewater (100 c.c.).

2. *Mucoid control.* 25 c.c. of the mucoid solution + 75 c.c. of water (100 c.c.).

3. *Glycogen-mucoid solutions:*

a. 40 c.c. glycogen + 25 c.c. mucoid + 35 c.c. water (100 c.c.).

b. 75 c.c. glycogen + 25 c.c. mucoid (100 c.c.).

On adding 2 c.c. of 0.8 per cent hydrochloric acid to each beaker, all but the glycogen control assumed a uniform milkiness. A second addition of 2 c.c. of the acid resulted in equal flocculent precipitation

TABLE VIII.  
NITROGEN CONTENT OF GELATINE-MUCOID PRECIPITATES.

SOLUTIONS.	CONTROLS.		MIXTURES.			
	Gelatine.	Mucoid.	Gelatine-mucoid.			
	1	2	3	4	5	6
	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.
Gelatine . . . .	80	..	10	20	40	80
Mucoid . . . .	..	50	50	50	50	50
Water . . . .	120	150	140	130	110	70
Total volume . .	200	200	200	200	200	200
ANALYTIC DATA (FOR DRY SUBSTANCE).						
Weight . . . .	gram	gram	gram	gram	gram	gram
	0.0	0.9862	1.0159	1.1720	1.3567	1.7872
Nitrogen content of the ash-free substance . . .	per cent	per cent	per cent	per cent	per cent	per cent
	..	13.29	13.77	13.85	14.13	14.48
Ash . . . .	..	2.23	1.86	1.34	1.48	1.21

in all but the glycogen control, which was unaffected. The original glycogen opalescence in 3, a and b, was quite as decided after the precipitation of the mucoid by the acid treatment as before. A total of 8 c.c. of acid was added to completely precipitate the mucoid in 2. The same amount was required for complete precipitation in the others. On standing, the glycogen opalescence in 1 and in the supernatant fluids in 3 was unchanged, and the precipitates failed to show a staircase effect. The following gravimetric data were obtained:

Controls.		3. Glycogen-Mucoid Mixtures. <sup>1</sup>	
1. Glycogen.	2. Mucoid.	a.	b.
0.0	0.1580 gm.	0.1610 gm.	0.1660 gm.

**Nitrogen content of proteid-mucoid precipitates.**— *With gelatine.*  
 XXXI. Mixed mucoid (30 grams, moist) was dissolved in 250 c.c. of 0.5 per cent potassium hydroxide. A 2 per cent solution of gelatine

TABLE IX.  
 NITROGEN CONTENT OF MUSCLE PROTEID-MUCOID PRECIPITATES.

SOLUTIONS.	CONTROLS.		MIXTURES.			
	Muscle extract.	Mucoid.	Muscle proteid-mucoid.			
	1	2	3	4	5	6
Muscle extract . . . .	c.c. 80	c.c. ..	c.c. 10	c.c. 20	c.c. 40	c.c. 80
Mucoid . . . . .	..	30	30	30	30	30
Water . . . . .	120	170	160	150	130	90
Total volume . . . .	200	200	200	200	200	200
ANALYTIC DATA (FOR DRY SUBSTANCE).						
Weight . . . . .	gram 0.0	gram 0.6576	gram 0.8348	gram 0.9787	gram 1.2658	gram 1.5238
Nitrogen content of the ash-free substance .	per cent ..	per cent 12.48	per cent 12.99	per cent 13.16	per cent 14.03	per cent 14.66
Ash . . . . .	..	0.36	0.17	0.33	0.31	0.51
SUBSTANCE PRECIPITATED FROM THE FILTRATES (180 C.C.) BY ALCOHOL (600 C.C.).						
Weight of dry substance	gram 0.8997	gram 0.0221	gram 0.0159	gram 0.0253	gram 0.0428	gram 0.2788

was used. 0.8 per cent hydrochloric acid was taken to precipitate the mixtures, the characters of which are indicated in Table VIII. 12 c.c. were required to completely precipitate the mucoid control. The same volume was finally added to each of the others. The precipitative effects noted in Experiments I-VII were again observed. The

<sup>1</sup> The precipitates were washed until the washings were free from opalescence and failed to give an iodine reaction.

precipitates were thoroughly and quantitatively washed by decantation in water until free from biuret reacting soluble matter, then in increasing strengths of alcohol, finally in ether, in preparation for analysis. Analytic data are summarized in Table VIII.

*With muscle proteids.* XXXII. 20 grams of moist mucoid from a second extract were dissolved in 150 c.c. of 0.5 per cent potassium hydroxide. A fairly concentrated watery extract of fresh hashed beef was made at 40° C. The extract was bloody. 0.8 per cent hydrochloric acid was used to precipitate, and 10 c.c. were added to each mixture referred to in Table IX. Equal volumes of the filtrates, 180 c.c., were treated in each case with 600 c.c. of 95 per cent alcohol, and the precipitates weighed. The decolorization, precipitative, and staircase effects noted in Experiments XI-XV were again observed.

*With egg albumen.* XXXIII. Mucoid from a second extract was dissolved in 0.5 per cent potassium hydroxide. The whites of three eggs were dissolved in 350 c.c. of water. 0.8 per cent hydrochloric acid was the precipitant. 17 c.c. of the acid were added in 5 c.c. volumes at intervals of ten minutes, until the mucoid controls and all mucoid-containing mixtures were completely precipitated. The precipitative effects, in the various mixtures indicated in Table IX, were relatively the same as those noted in Experiments XX-XXIX.

Analytic data are summarized in Table IX.

#### GENERAL PROPERTIES OF THE PROTEID-MUCOID PRECIPITATES.

Most of the precipitates resembled precipitated tendomucoid. The gelatine-mucoid precipitates usually manifested a gelatinous consistence, and frequently resembled aluminium hydroxide.

The meat extract-mucoid precipitates were pigmented by hæmoglobin products. That the pigment was not held mechanically in the precipitates was shown by the fact that the color could not be washed out. We have already alluded to the fact that the filtrates from the precipitates were colorless, except when too much blood was present in the meat extract to begin with. Such decolorization fails to occur when a washed flocculent mucoid precipitate is thoroughly stirred in a neutral or alkaline bloody meat extract. Under such conditions, also, the precipitate remains snow white. It is probable, therefore, that in the presence of acid, mucoid and hæmoglobin unite chemically.

In all cases the proteid-mucoid precipitates not only weighed more than the mucoid controls, but were obviously more bulky, even on

long standing, as well as under conditions favoring rapid sedimentation of each precipitate. While our precipitates were being washed we occasionally observed that the washings gave a precipitate on mixing with the acid filtrate. It is probable that our products varied in their solubility in dilute acid and that more accurate knowledge of the conditions favoring their formation might give even more decided gravimetric results than those already obtained.

All of the thoroughly washed and purified products<sup>1</sup> were acid in

TABLE X.

NITROGEN CONTENT OF EGG ALBUMEN-MUCOID PRECIPITATES.

SOLUTIONS.	CONTROLS.				MIXTURES.			
	Egg albumen.		Mucoid.		Egg albumen-mucoid.			
	1	2	3	4	5	6	7	8
Egg albumen . . . . .	c.c. 50	c.c. 50	c.c. ..	c.c. ..	c.c. 10	c.c. 20	c.c. 40	c.c. 80
Mucoid . . . . .	..	..	50	50	50	50	50	50
Water . . . . .	150	150	150	150	140	130	110	70
Total volume . . . . .	200	200	200	200	200	200	200	200
ANALYTIC DATA (FOR DRY SUBSTANCE).								
Weight . . . . .	gram 0.0	gram 0.0	gram 1.227	gram 1.214	gram 1.382	gram 1.542	gram 1.614	gram 1.726
Nitrogen content of ash-free substance . . . .	per cent ..	per cent ..	per cent 11.95	per cent 11.89	per cent 12.12	per cent 12.35	per cent 12.46	per cent 12.57
Ash . . . . .	..	..	0.56	0.49	0.59	0.35	0.27	0.32

reaction, though the degree of acidity varied considerably; they yielded reducing substance and glucothionic acid on decomposition with dilute hydrochloric acid, and gave the common mucoid reactions as distinctly as the mucoids themselves.

When the precipitates were dissolved in dilute limewater to neutral solutions, the latter failed to become turbid, or even opalescent, on boiling. The entire series of products obtained in Experiment XXIII

<sup>1</sup> Purified by the customary methods of washing in water, alcohol and ether.

was carefully studied in this respect, with the negative results just stated.

On acidification of the neutral or alkaline solutions of the proteid-mucoid products, they were immediately rendered milky, and a fine flocculent precipitate quickly separated. The resistance of these compounds to the solvent action of acid is quite as marked, in most cases, as that of the mucoids themselves.

#### DISCUSSION OF RESULTS.

Our results support the belief that the mucoids have the power in acid media of uniting chemically with various proteids. Acid precipitation of mucoid from solutions containing other proteids, such as alkaline tendon extracts, seems to result in the removal of portions of these proteids in chemical combination with the mucoid and, also, in the formation of products which had no previous existence. This fact would account for the wide discrepancies in the past among the results of elementary analysis of the mucoids. Our observations indicate, therefore, that previous quantitative determinations of mucoids in tissues or proteid fluids have been inaccurate, and that new methods must be devised for such analyses. Even if our products were only intimate mechanical admixtures, the latter observations would hold good, for we have seen that the associated proteid cannot be washed out by the methods now used for mucoid purification.

Our proteid-mucoid products seemed to require less acid for their precipitation than equivalent amounts of mucoid in equal volumes under equal conditions of reaction, etc. In other words, the proteid-mucoid products are more easily precipitated.

That the precipitates formed in these experiments are not merely mechanical mixtures is indicated by the facts on their general properties referred to on page 426. In Experiment XXVIII it was clearly shown that precipitated and washed mucoid may be mixed with neutral or faintly alkaline egg albumen solutions in large excess without holding any of the latter that could not be washed out readily with water (compare 2 and 3). In Experiment XXVIII we also saw, however, that in an acid mucoid mixture instant increase of the flocculent precipitate occurred when albumen solution was added (5), although the same amount of acid had no precipitative effect on a control albumen solution.<sup>1</sup> The weight of precipitate formed

<sup>1</sup> This fact indicates that the union of mucoid and proteid *in the presence of acid* results not only from a consequent chemical change in the mucoid salt, but also



under such conditions (5) was practically the same, also, as that produced when an alkaline mixture of equal amounts of mucoid and albumen was acidified (6). The data in Experiment XXVII also harmonize with these deductions. See Table VII.

It might be assumed that alkali albuminate was formed from the proteid associated with the mucoid, when their solutions were mixed. But our precipitation results cannot be attributed to admixture of mucoid with insoluble albuminate. In some of the experiments a very slight precipitate of albuminate may have been retained by the mucoid compound. The quantity of precipitate in the associated proteid control at the end of each experiment shows, however, that any such admixture was trivial in amount. (See especially Experiments II, XVIII, XX, XXI, and XXIV to XXVII.) Our results with gelatine are particularly conclusive in this connection.

The increase in the weights of the proteid-mucoid precipitates has been somewhat irregular, but the conditions of our experiments have not favored accurate estimation of relative combining powers with the various proteids employed. The degree of acidity, for example, was never the same in connection with the formation of our products. Of the various proteids employed, proteose (Witte's "peptone") was combined in the smallest proportion (by weight). The proteids of higher molecular weight were taken up in the largest proportion (by weight). Molecular proportions in the compounds have not yet been investigated.

That mucoid does not unite with all colloids, under circumstances similar to those in our experiments, and that the mere colloidal condition of the associated substance is not responsible for the effects noted, is indicated by the negative results with glycogen (Experiment XXX).

Differences in ash content were without influence (Experiments XIII, XXXI, XXXII, XXXIII).

Our results for nitrogen content of the mucoids and proteid-mucoid compounds harmonize with those for relative weights of the precipitates, and for the quantities of associated proteid matter remaining in the filtrates. They also remind us of the difficulties alluded to on the next page.

With reference to the divergent data for elementary composition

in the associated proteid. *Combined* acid also appears to have such influences (Experiment X).

of their tendomucoid products, Chittenden and Gies<sup>1</sup> stated, "Our results seemingly justify the assumption that white fibrous connective tissue contains more than one mucin, or else that the mucin obtainable from this tissue is prone to carry with it a certain amount of some other form of proteid matter which the ordinary methods of purification are not wholly adequate to remove. . . . Undoubtedly preliminary extraction of the tissue with salt solution tends to remove a certain amount of proteid matter, especially globulins, which might otherwise render the product impure. . . . Still there is no certainty on this point, for it is to be remembered that precipitation of the mucin requires the addition of considerable hydrochloric acid beyond neutralization of the alkaline fluid, and this excess of acid would naturally exert a marked solvent action upon any albuminous matter present."<sup>2</sup> Chittenden and Gies were considering the possibility that *mechanical* admixture of associated proteid might have effected the divergences among their figures for elementary composition, although the latter part of the quotation above indicates that in their belief the other alternative proposed — existence of several mucoids in tendon — accounted for the analytic variations observed.

A few years later, Cutter and Gies<sup>3</sup> studied, by improved methods, the question of tendomucoid composition, and found that the mucoids from five successive extracts of the same tendon pieces exhibited marked, though fairly regular, differences in elementary composition. In this connection they wrote as follows: "We can no longer believe that proteid impurity is responsible for the observed variations. In the first place, the quantity of soluble proteid in tendon, other than mucoid, is very slight. Experiments in progress in this laboratory indicate that it is less than 0.3 per cent.<sup>4</sup> If, however, it were possible for all of this small quantity to combine permanently with the precipitated mucoids, it could not account for the regular rise and fall of nitrogen content observed in each series of our experiments. Although it is conceivable that the mucoid of the first extract could be so affected, such an assumption would not explain the rise of nitrogen

<sup>1</sup> CHITTENDEN and GIES: *Journal of experimental medicine*, 1896, i, p. 194; also GIES and COLLABORATORS: *Biochemical researches*, 1903, i, Reprint No. 13, p. 205.

<sup>2</sup> *Id.* *ib.* are our own.

<sup>3</sup> CUTTER and GIES: *Loc. cit.*

<sup>4</sup> BEERGER and GIES: *This journal*, 1901, vi, p. 228; also *Biochemical researches*, 1903, i, Reprint No. 8.

in the third and subsequent extracts, particularly in view of the marked fall of the same in the second. Then, too, each product was so thoroughly washed in excess of 0.2 per cent hydrochloric acid that unless very intimate and unusual chemical union resulted, lymph proteids must have been quickly and completely dissolved from the precipitates. We know of no other substance in tendon which would resist the washing treatment and, by mechanical admixture or chemical combination, account for the orderly variations observed in the analytic series."

Shortly before the publication of the paper by Cutter and Gies, we together began a new research to ascertain, if possible, additional reasons for the variations in the analytic data for the glucoproteids. Nerking<sup>1</sup> had just published his paper on "fat-proteid compounds." His results and views made it seem possible that the variations noted in the analytic results for mucoids, as well as other proteids, were due, perhaps in part at least, to combinations or intimate admixtures of the proteid substance with fat or fatty acid. It has been known for a long time that a certain amount of ether-soluble matter is associated with crude connective tissue mucoid.<sup>2</sup> The difficulties in the way of removing this admixture have been appreciated by various observers, and as no one had determined the chemical nature of this extractive matter, its existence appeared to be significant in this connection.

Our investigation demonstrated,<sup>3</sup> however, that the glucoproteids as commonly prepared are not fat compounds, that they bear no resemblance to lecithalbumins and that the analytic data for *purified* mucoids are not affected by fatty radicles. The results of the present research, however, indicate the nature of one, perhaps *the*, disturbing factor in mucoid preparation and analysis.

That the mucoid from the *first* extract of such a tissue as tendon is seriously affected by associated lymph proteids, when the extract is acidified, is now definitely shown. Our results appear to offer no explanation, however, for the observation of Cutter and Gies, and also our own in another connection,<sup>4</sup> that the nitrogen content of mucoids from the third and successive extracts rises steadily. Cutter and Gies also found that each successive mucoid extract required more and

<sup>1</sup> NERKING: Archiv für die gesammte Physiologie, 1901, lxxxv, p. 330.

<sup>2</sup> LOEBISCH: Zeitschrift für physiologische Chemie, 1886, x, p. 58.

<sup>3</sup> POSNER and GIES: This journal, 1902, vii, p. 331; also Biochemical researches, 1903, i, Reprint No. 35.

<sup>4</sup> POSNER and GIES: This journal, 1904, xi, p. 335.

more acid for its precipitation. Our own observations suggest that this result may have been due to a decreasing proportion of associated lymph proteids in the successive extracts. It may be, also, that the increasing strength of acid, which is required to precipitate the successive extracts, transforms the products sufficiently to account for the steady rise in nitrogen content.<sup>1</sup>

Our data further indicate that the mucoids may exist in the tissues in combination with other proteids. This view is borne out by the fact that mucoids cannot be satisfactorily extracted from the connective tissues with water or neutral saline fluids, although neutral mucoid (*e.g.*, potassio-mucoid) readily dissolves in such solutions. It is possible that dilute alkalis, which are so well-adapted for the extraction of the mucoids, in such cases break the glucoproteid from complex proteid (protoplasmic) combinations, and, besides, furnish the cations for the formation of easily soluble ion-mucoid products in the extract. These observations are in accord with the remarks of Schmiedeberg on the forms in which chondroitin sulphuric acid may exist in cartilage and on the varieties of chondromucoid-like substances (peptochondrin, chondralbumin, etc.) obtainable from that tissue.<sup>2</sup>

The behavior of other glucoproteids, under circumstances similar to and also different from the conditions in these experiments, will shortly be ascertained. Whether nucleoproteids show similar combining properties will also be investigated.

#### SUMMARY OF GENERAL CONCLUSIONS.

In the presence of acid, tendomucoid forms relatively insoluble compounds with gelatine, proteoses, alkali albuminate, acidalbumin, and with proteids in aqueous extracts of muscle and tendon, and in blood serum and white of egg. In these respects, mucoid behaves much like chondroitin sulphuric acid, and the glucothionic acids from various glucoproteids. Consequently, when mucoid is precipitated with acid from neutral or alkaline solutions, such as tendon and other tissue extracts, non-mucoid-proteid is withdrawn from the fluid in proteid-mucoid combination. This fact makes it evident that the methods for

<sup>1</sup> This matter is now under investigation in this laboratory.

<sup>2</sup> SCHMIEDEBERG: *Archiv für experimentelle Pathologie und Pharmakologie*, 1891, xxviii, p. 399. See also a recent paper by MÖRNER, on pericaglobulin: *Zeitschrift für physiologische Chemie*, 1904, xl, p. 451. This important paper appeared since the completion of our experiments.

determining the quantities of mucoids in tissues, etc., have heretofore been inaccurate, and that many so-called "pure" mucoids have been proteid-mucoid products.

Hydrochloric acid, sulphuric acid, and acetic acid manifested the same general precipitative influence on proteid-mucoid mixtures. Combined hydrochloric acid also exerted a precipitative effect.

When all other conditions were equal, precipitation usually occurred first in the solutions having the greatest proportion of associated proteid. The extent of this precipitative influence has not yet been ascertained. The proteid-mucoid products were more easily precipitated with acid than the mucoid itself.

Precipitates of proteid-mucoid may be formed by treating the neutral or alkaline mixture of the two with acid, or by adding the neutral or faintly alkaline fluid of the one to an acidified solution of the other. Even *solid, freshly precipitated* mucoid combines with proteid when it is mixed with a solution of the latter in the presence of acid.

In these unions of mucoid with proteid it is probable that the chemical conditions of both the mucoid and the associated proteid are changed by the acid in such ways as to favor combination of the two. Mechanical admixture does not seem to account for the results obtained.

The proteid-mucoid products which were formed in these experiments possessed the general properties of the original tendomucoid. They were acid in reaction, relatively insoluble in dilute acid, yielded reducing substance and glucothionic acid on decomposition in dilute hydrochloric acid, gave the common mucoid precipitation reactions, neutralized dilute alkaline fluids, and were non-coagulable in neutral ion-solution. The physical properties of the freshly precipitated products were occasionally unlike those of tendomucoid. Thus the gelatine-mucoid was somewhat gelatinous, resembling freshly-precipitated aluminium hydroxide.

The nitrogen content of the proteid-mucoid compounds was always higher than that of the tendomucoid itself. There were no particular differences in ash-content. It is very probable that divergences among the results for elementary composition of the mucoids have been due heretofore to conditions which favored the production of compounds containing variable proportions of associated proteid matter.

It is probable, also, that the mucoids exist in the tissues in complex

and relatively insoluble combinations, such as might be formed with albuminous bodies. That the mucoids in the tissues are not merely simple neutral or alkaline ion-compounds, is shown by the fact that alkaline solution is required to extract them satisfactorily. The alkali used to effect extraction probably breaks up such proteid-mucoid combinations, converting the mucoid into ion-products that are readily soluble, even in water.

The mere colloidal condition of the proteids does not seem to have accounted for the results obtained. Under conditions that would have favored the formation of proteid-mucoid compounds, glycogen failed to unite with the glucoproteid.

## THE AUTOLYSIS OF ANIMAL ORGANS.

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### I. THE END-PRODUCTS OF THE AUTOLYSIS OF THE TESTES.

**T**HIS communication is a continuation of the work published in the *Zeitschrift für physiologische Chemie*, under the title: "Die Endprodukte der Selbstverdauung tierischer Organe."

The importance of autolysis as a possible explanation of the process of animal metabolism was indicated in that article, and it will suffice, therefore, to report here the analysis of the crystalline end-products of autolysis of the glands in question.

Following the plan of the work on the pancreas and the liver, attention was directed to the amino-acids, hexon bases, purin and pyrimidin bases.

In a preliminary experiment it was found that the autolysis of the testes is similar to that of other organs, — most active in a solution of 0.2 per cent acetic acid. The glands were minced and allowed to stand twenty-four hours at room-temperature in 0.2 per cent of the acid. Sufficient toluol and chloroform were added to prevent bacterial growth. After twenty-four hours the extracts were filtered through cloth, and allowed to stand at room-temperature for nine months. Care was taken that the extracts had enough toluol and chloroform to prevent putrefaction. At the end of that time the solutions were neutralized, heated, filtered, and concentrated on the water bath until the beginning of crystal formation.

**Tyrosin.** — Part of the crystalline mass thus formed was dissolved in hot diluted ammonia water and decolorized by means of charcoal. The solution was rendered acid by means of acetic acid while hot, and filtered. On cooling, tyrosin separated in fine white needles. Washed with alcohol and ether, and dried on toluol bath, the crystals showed a melting point of 315° C. (not corr.).

0.1035 gram of the substance gave, on combustion, 0.2270 gram  $\text{CO}_2$  and 0.0575 gram  $\text{H}_2\text{O}$ .

For  $\text{C}_9\text{H}_{11}\text{NO}_3$ :

Calculated:	C —59.66 per cent	H —6.07 per cent.
Found:	C —59.81 “	H —6.17 “

The remainder of the product of self-digestion was divided into two parts, the smaller one for analysis of the basic products, and the larger part for that of the amino-acids.

**Amino-acids.** — For the purpose of obtaining the amino-acids, the mixture was concentrated on water bath to dryness. To remove as much of the adhering water as possible, it was repeatedly evaporated with 95 per cent alcohol, and finally taken up with absolute alcohol, and the acids transformed into their esters by Fischer's process. By this process the hydrochloric salts of the esters were formed, and these were transformed into the free esters, also by the method of Fischer. The mixture of esters was divided by means of distillation at a pressure of 9 mm. into the following fractions:

Boiling at 50° C.	20.0 grams.
50°–75° C.	25.0 “
75°–90° C.	49.5 “
90°–105° C.	20.0 “
105°–135° C.	26.8 “
135°–155° C.	22.5 “

Another lot of the esters gave the following fractions:

At 50° C.	13.5 grams.
50°–75° C.	18.0 “
75°–90° C.	24.0 “
90°–105° C.	16.5 “
105°–160° C.	38.5 “

All the fractions distilling below 105° C. were saponified by boiling with distilled water, those distilling above that temperature by baryta water or by strong hydrochloric acid.

*Fraction 50° C.* — It contained chiefly alanin and the other lower amino-acids.

The fraction was saponified and evaporated to dryness. The dry residue was extracted with absolute alcohol, and the alcoholic residue dissolved in a little boiling water. On standing, there formed only a very slight crystalline precipitate; to the mother liquid absolute alcohol was added in quantity sufficient to produce turbidity. It



was then allowed to crystallize. The substance thus formed was recrystallized out of diluted alcohol, and on analysis proved to consist of alanin contaminated slightly with other amino-acids. The mother liquid from the alcoholic precipitate was treated with ether, and the sediment thus formed dissolved in hot water; to this solution alcohol was added until the clear solution became turbid. On standing, there formed a crystalline deposit. This was filtered, washed with absolute alcohol and ether, dried in vacuum desiccator over sulphuric acid, and finally in xylol bath.

The substance had the following composition:

0.1734 gm. gave, on combustion, 0.2560 gm. of  $\text{CO}_2$  and 0.1186 gm. of  $\text{H}_2\text{O}$ .

For  $\text{C}_3\text{H}_5\text{O}_2\text{N}$ :

Calculated:	C —40.45 per cent	H —7.87 per cent.
Found:	C —40.37 “	H —7.59 “

*Fraction 50°–75° C.* — This was saponified like the previous fraction, evaporated to dryness, and extracted with alcohol. The residue was dissolved in just enough boiling water to accomplish solution. It was then allowed to stand twenty-four hours. There formed a crystalline precipitate consisting chiefly of leucin. The mother liquid was treated with copper oxide, filtered, the filtrate concentrated on water bath until it began to crystallize. It was then allowed to stand twenty-four hours, and filtered. The filtrate was again concentrated to a syrup, and again allowed to stand over night. There formed a second precipitate. This second precipitate was redissolved in an excess of boiling distilled water. The solution was allowed to stand over night and filtered. The filtrate was again concentrated. The precipitate then formed was filtered, washed with alcohol and ether, dried in vacuum desiccator over sulphuric acid, and finally in toluol bath.

It had the following composition:

0.2029 gm. of the substance gave, on combustion, 0.2661 gm.  $\text{CO}_2$  and 0.1075 gm.  $\text{H}_2\text{O}$  and 0.0614 gm.  $\text{CuO}$ .

For  $(\text{C}_4\text{H}_8\text{O}_2\text{N})_2\text{Cu}$ :

Calculated:	C —35.95 per cent	H —5.99 per cent	Cu —23.59 per cent.
Found:	C —35.78 “	H —5.88 “	Cu —24.16 “

The substance thus has the composition of the copper salt of an aminobutyric acid. Aminobutyric acid has not as yet been identified

among the decomposition products of proteid material, and therefore further investigation is necessary in order to establish the true nature of this substance.

One other observation makes it probable, however, that the substance was an aminobutyric acid. The mother liquid of the copper salt, consisting apparently of copper salts of some amino-acids soluble in water, was joined to an analogous solution obtained from the next fraction, and then treated with alcohol. On prolonged standing there formed a precipitate which was redissolved in very little hot water, and alcohol was added until the clear solution just began to turn turbid. On standing, a precipitate again formed, which was washed and dried in the usual manner. It had the following composition :

0.1340 gm. of the substance gave 0.1746 gm.  $\text{CO}_2$ , and 0.0725 gm.  $\text{H}_2\text{O}$ .

For  $(\text{C}_4\text{H}_8\text{O}_2\text{N})_2 \text{Cu}$  :

Calculated :	C —35.95 per cent	H —5.99 per cent.
Found :	C —35.65 “	H —6.01 “

Here again we have the composition of a copper salt of aminobutyric acid.

*Fraction 75°–90° C.* — This was treated in exactly the same manner as the previous fraction. The second copper salt was analyzed with the following result :

0.1930 gm. of the substance gave, on combustion, 0.2895 gm. of  $\text{CO}_2$ , 0.1230 gm. of  $\text{H}_2\text{O}$ , and 0.0575 gm. of  $\text{CuO}$ .

For  $(\text{C}_5\text{H}_{10}\text{O}_2\text{N})_2 \text{Cu}$  :

Calculated :	C —40.68 per cent	H —6.78 per cent	Cu —21.35 per cent.
Found :	C —40.91 “	H —7.07 “	Cu —21.26 “

The substance thus had the composition of the copper salt of amino-valerianic acid.

*Fraction 90°–105° C.* — This fraction was saponified, evaporated to dryness, and extracted with absolute alcohol like the previous one. It contained chiefly leucin, and also aminovalerianic, and perhaps the lower acids. The most insoluble part of the fraction was twice recrystallized out of water, washed with alcohol and ether, and dried in xylol bath. It had a melting point of  $295^\circ \text{C}$ . (not corr.), and the following composition: 0.2100 gm. of the substance gave, on combustion, 0.4235 gm.  $\text{CO}_2$ , and 0.13870 gm.  $\text{H}_2\text{O}$ .

For  $C_6H_{13}O_2N$ :

Calculated:	C —54.96 per cent	H —9.92 per cent.
Found:	C —55.00     "	H —9.89     "

**Pyrrolidincarboxylic acid.** — The combined alcoholic extracts of all the fractions were evaporated to dryness and the residue extracted with hot absolute alcohol. On cooling there formed a precipitate, which was removed by filtration, and the filtrate again evaporated to dryness. The operation of evaporating and extracting the residue with hot absolute alcohol was repeated several times until a residue was obtained which was entirely soluble in absolute alcohol. The last residue was dissolved in water and treated with copper oxide. The filtrate was evaporated to dryness, and the residue extracted with hot absolute alcohol. Part of it remained insoluble in alcohol. However, it did not have the properties of the copper salt of the inactive pyrrolidincarboxylic acid. It crystallized from aqueous solution without water of crystallization, and had the following composition:

0.1265 gm. gave, on combustion, 0.1820 gm. of  $CO_2$ , and 0.0698 gm. of  $H_2O$ , and 0.0335 gm. of  $CuO$ .

For  $(C_5H_8O_2N)_2 Cu$ :

Calculated:	C —41.24 per cent	H —5.50 per cent	Cu —21.55 per cent.
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For  $(C_5H_{10}O_2N)_2 Cu$ :

Calculated:	C —40.68 per cent	H —6.78 per cent	Cu —21.35 per cent.
Found:	C —39.24     "	H —6.14     "	Cu —21.14     "

The substance consisted apparently of aminovalerianic acid. The alcohol soluble part was evaporated to dryness, dissolved in water, and treated with sulphureted hydrogen. The filtrate was evaporated to dryness and the residue taken up in hot alcohol. However, it was impossible to crystallize the active  $\alpha$ -pyrrolidincarboxylic acid from this solution. It is, however, possible that the acid was present in very small quantities, and that the impurities present in the residue hindered its crystallization.

*Fractions 105°–130° C. and 130°–160° C.* — These fractions contained the dibasic amino-acids and phenylalanin. The phenylalanin was separated from the other acids according to Fischer's method in the following manner: The esters were taken up in ether and repeatedly shaken in a separatory funnel with distilled water. The

phenylalanin ester thus remains in the ethereal solution, being insoluble in water, while the other esters are in the aqueous solution.

**Phenylalanin.**—The ethereal solution was taken up with strong hydrochloric acid, and the ether allowed to evaporate at low temperature. On prolonged standing, crystals of the hydrochloric salt of phenylalanin separated out. These were removed from the mother liquid on suction funnel, and dissolved in water containing an excess of ammonia. This solution was then evaporated nearly to dryness, allowed to cool, and filtered on suction funnel. The residue was twice recrystallized from water.

The substance thus obtained and dried in xylol bath had the following composition:

0.1420 gm. of the substance gave, on combustion, 0.3380 gm.  $\text{CO}_2$ , and 0.0840 gm.  $\text{H}_2\text{O}$ .

For  $\text{C}_{10}\text{H}_{11}\text{O}_2\text{N}$ :

Calculated:	C —65.45 per cent	H —6.66 per cent.
Found:	C —64.91 “	H —6.57 “

**Aspartic acid.**—The aqueous solution containing the dibasic acid was saponified by heating on the water bath with barium hydrate and water. On cooling, the basic barium salt of aspartic acid separated out. The barium was then removed quantitatively by sulphuric acid, and the solution of the free acid concentrated at diminished pressure until it began to crystallize. The acid was recrystallized from water. For analysis the substance was dried in xylol bath.

0.1085 gm. of the substance gave, on combustion, 0.146 gm. of  $\text{CO}_2$  and 0.0540 gm. of  $\text{H}_2\text{O}$ .

For  $\text{C}_4\text{H}_7\text{O}_4\text{N}$ :

Calculated:	C —36.09 per cent	H —5.26 per cent.
Found:	C —36.62 “	H —5.53 “

Glutamic acid was obtained from the filtrate of the basic barium salt of aspartic acid. The barium was removed quantitatively by sulphuric acid, and the filtrate from barium sulphate concentrated to dryness at diminished pressure. The residue was dissolved in strong hydrochloric acid, and through this solution dry hydrochloric acid was passed. The solution was kept in cooling mixture during the operation. Only on prolonged standing did a crystalline sediment appear. This was re-crystallized from water, washed with cold absolute alcohol, and saturated with dry hydrochloric acid. The

yield of the substance thus obtained was very small. It had the following composition:

0.1710 gm. of the substance gave, on combustion, 0.2150 gm. of  $\text{CO}_2$  and 0.0775 gm. of  $\text{H}_2\text{O}$ .

For  $\text{C}_5\text{H}_9\text{O}_4\text{NHCl}$ :

Calculated:	C —32.75 per cent	H —5.40 per cent.
Found:	C —32.63    "	H —5.06    "

**Purin bases.**—The purin bases were separated by means of Hopkins' solution of mercuric sulphate; the precipitate thus obtained was decomposed by sulphureted hydrogen. The adenin and guanin fractions were so small that the substances could not be purified for analysis. The hypoxanthin was transformed into the silver nitrate, and dried in a vacuum desiccator over sulphuric acid.

0.1345 gm. of substance gave 0.0470 gm. Ag.

For  $\text{C}_5\text{H}_4\text{N}_4\text{O}$ . Ag  $\text{N.O}_3$ :

Calculated:	Ag —35.26 per cent.
Found:	Ag —34.93    "

The filtrate from hypoxanthin silver nitrate gave, on addition of ammonia, a flocculent precipitate which was not analyzed.

**Pyrimidin and hexon bases.**—It was attempted to separate the pyrimidin and hexon bases by means of Hopkins' mercuric sulphate solution. Kossel had already used this reagent for separating cytosin, and also for purifying histidin. The application of the reagent for the separation of the three "hexon" bases resulting from acid hydrolysis of proteids will be communicated later. Here it will suffice to state that it was impossible to demonstrate by this process the presence of either the pyrimidin bases or of arginin and histidin. It is impossible to say at present whether or not the substances are actually absent among the end-products of the autolysis of testes. Investigations of this problem are in progress. Equally futile were the attempts to obtain lysin. The filtrate from arginin and histidin fractions was freed from mercury, and the filtrate treated with phosphotungtic acid. The precipitate thus formed was decomposed in the usual manner, and the solution freed from phosphotungtic acid, concentrated to the thickness of a syrup, and treated with an alcoholic solution of picric acid. The precipitate thus formed consisted chiefly of ammonium picrate. The mother liquor was freed from picric acid by means of sulphuric acid and ether. The sulphuric acid was then

removed by means of barium hydrate, and the filtrate from the barium sulphate concentrated to a thick syrup. On standing, it solidified to a semi-crystalline mass, soluble in methyl alcohol, and insoluble in ethyl alcohol. The substance turned red in presence of alkali, and very light yellow on neutralization. The nature of the substance is not as yet established. Thus the presence of lysin among the end-products of the autolysis of the testes remains uncertain. Further investigations in that direction are in progress.

## II. THE END-PRODUCTS OF THE AUTOLYSIS OF THE SPLEEN.

The end-products of the autolysis of the spleen were investigated recently by Leathes. The number of amino-acids identified by him was, however, few, and those that had been separated were obtained in very small quantities. In the present paper, there will be communicated only the results of the analysis of the amino-acids appearing on autolysis of the gland. The methods employed in the work were exactly the same as those employed for the analysis of the substance obtained under similar conditions from the testes.

The following fractions of the esters were obtained:

50° C.	—12.0	grams.
50–80° C.	—18.0	“
80–100° C.	—26.5	“
100–130° C.	—11.5	“
130–160° C.	—14.5	“

*Fraction under 50° C.* — This consisted chiefly of alanin, and perhaps of the other lower acids.

The substance used for analysis had the following composition:

0.1360 gm. of the substance 0.2030 gm. CO<sub>2</sub> and 0.0963 gm. H<sub>2</sub>O.

For C<sub>3</sub>H<sub>7</sub>O<sub>2</sub>N:

Calculated:	C —40.45 per cent	H —7.87 per cent.
Found:	C —40.70 “	H —7.83 “

*Fraction 50° — 80° C.* — This fraction consisted of leucin, amino-valerianic, and aminobutyric acids.

The copper salt of the aminobutyric acid used for analysis had the following composition:

0.1935 gm. of the substance gave, on combustion, 0.2515 gm. CO<sub>2</sub>, 0.1000 gm. of H<sub>2</sub>O and 0.0582 gm. of CuO.

For  $(C_4H_8O_2N)_2 Cu$ :

Calculated:	C -35.95 per cent	H -5.99 per cent	Cu -23.59 per cent.
Found:	C -35.44 "	H -5.83 "	Cu -24.02 "

*Fraction 80° - 100° C.* — This consisted chiefly of leucin and amino-valerianic acid.

The copper salt of the aminovalerianic acid used for analysis had the following composition:

0.1590 gm. of the substance gave 0.2410 gm.  $CO_2$ , 0.0976 gm.  $H_2O$ , and 0.0425 gm.  $CuO$ .

For  $(C_5H_{10}O_2N)_2 Cu$ :

Calculated:	C -40.68 per cent	H -6.78 per cent	Cu -21.35 per cent.
Found:	C -41.33 "	H -6.81 "	Cu -21.34 "

*Fraction 80° - 100° C.* — This fraction consisted chiefly of leucin and aminovalerianic acid. Only the most insoluble part of the fraction was analyzed. It had the following composition:

0.2080 gm. of the substance gave, on combustion, 0.4165 gm. of leucin and 0.1835 gm. of  $H_2O$ .

For  $C_6H_{13}O_2N$ :

Calculated:	C -54.96 per cent	H -9.92 per cent.
Found:	C -54.61 "	H -9.80 "

*$\alpha$ -Pyrrolidincarboxylic acid.* — The alcoholic extracts were treated in the manner already described. An alcohol soluble and insoluble copper salt were obtained. The alcohol insoluble salt did not have the properties of the pyrrolidincarboxylic acid. On prolonged standing of the alcoholic solution of the other fraction a very small precipitate appeared which had all the characteristic properties of the inactive  $\alpha$ -pyrrolidincarboxylic acid. The blue salt turned purple on drying, gave the pyrrol color test, and developed on heating the odor of pyrrolidin. There was, therefore, no doubt that pyrrolidincarboxylic acid was present this time among the end-products of autolysis of the spleen. There was not enough of the substance for analysis. I wish to remark here that several years ago I obtained a similar result while working on the end-products of autolysis of the pancreas. Repeating the experiments later, I failed, however, to find the substance. I also failed to find the acid on self-digestion of other glands. It seems at present that pyrrolidincarboxylic may be found in very small quantity among the products of autolysis.

The conditions necessary for its appearance will have to be studied in greater detail, the pyrrol being of great importance for the economy of the organism.

*Fractions 100°-130° and 130°-160° C.* — Phenylalanin, aspartic, and glutamic acids were identified in these fractions. The analysis of phenylalanin gave the following results:

0.1718 gm. of the substance gave, on combustion, 0.3785 gm. of  $\text{CO}_2$ , and 0.1020 gm. of  $\text{H}_2\text{O}$ .

For  $\text{C}_9\text{H}_{11}\text{O}_2\text{N}$ :

Calculated:	C -65.45 per cent	H -6.66 per cent.
Found:	C -65.36    "	H -6.45    "

The analysis of the aspartic acid gave the following results:

0.1230 gm. of the substance gave 0.1650 gm. of  $\text{CO}_2$  and 0.058 gm. of  $\text{H}_2\text{O}$ .

For  $\text{C}_4\text{H}_7\text{O}_4\text{N}$ :

Calculated:	C -36.09 per cent	H -5.26 per cent.
Found:	C -36.58    "	H -5.25    "

The analysis of the hydrochloric acid salt of the glutamic acid gave the following results:

0.1255 gm. of the substance gave, on analysis, the following results:

0.1255 gm. of the substance gave, on combustion, 0.1520 gm. of  $\text{CO}_2$ , and 0.0530 gm. of  $\text{H}_2\text{O}$ .

For  $\text{C}_5\text{H}_9\text{O}_4\text{NHCl}$ :

Calculated:	C -32.75 per cent	H -5.40 per cent.
Found:	C -33.03    "	H -5.59    "

*Tyrosin* was also obtained in the same manner as in the analysis of the end-products of self-digestion of the testes. The analysis gave the following results:

0.1500 gm. of the substance gave, on combustion, 0.3268 gm. of  $\text{CO}_2$  and 0.845 gm. of  $\text{H}_2\text{O}$ .

For  $\text{C}_9\text{H}_{11}\text{O}_3\text{N}$ :

Calculated:	C -59.66 per cent	H -6.07 per cent.
Found:	C -59.42    "	H -6.25    "

Thus all the animal glands so far studied form, on prolonged self-digestion, similar end-products. There seems, however, to be a marked difference in the proportions of the various substances occurring during the autolysis of different glands. This will be, however, the topic of special investigation.



I wish to express my indebtedness to Prof. R. H. Chittenden, under whose control this work was done. The expenses of the investigation were covered by a grant from the Carnegie Institution.

REFERENCES.

- KOSSEL and PATTEN : *Zeitschrift für physiologische Chemie*, 1903, xxxviii, p. 39.  
LEATHES : *Journal of physiology*, 1903, xxviii, p. 360.  
LEVENE : *Zeitschrift für physiologische Chemie*, 1904, xli, p. 393.



## THE EFFECT OF SUPRARENAL EXTRACT UPON THE PUPILS OF FROGS.

BY S. J. MELTZER AND CLARA MELTZER AUER.

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SUBCUTANEOUS injections of suprarenal extract have no effect upon the width of the pupils in mammals. Neither do instillations into the conjunctival sac exert any influence. These facts were established by Lewandowsky and many other investigators. In our recent study upon rabbits and cats,<sup>1</sup> we have established that subcutaneous injections, as well as instillations of adrenalin, readily cause a dilatation of the pupil, when the corresponding superior cervical ganglion has been previously removed. On the other hand, we have amply confirmed the statements of previous writers that in normal animals neither instillations nor subcutaneous injections exert any effect, no matter how large a dose was administered. And even after a previous section of the sympathetic nerve, which has a distinctly favoring effect upon the constriction of the blood-vessels, such administration of adrenalin exerts hardly any influence upon the dilatation of pupil. The only method of application which causes a dilatation of the pupil in normal mammals is intravenous injection. But even here the dilatation lasts less than one minute.

In the further pursuance of our studies, we now find that in normal frogs a subcutaneous injection of adrenalin readily causes a dilatation of the pupils which lasts longer than we have ever observed in mammals, even after removal of the superior cervical ganglion. The same is true also of instillations into the conjunctival sac.

The pupil in frogs is of an oval shape, with the long axis horizontal and the anterior pole somewhat angular. There is a slight notch on the lower border. The iris stands out very prominently between the black pupil and the yellowish white sclera; the pupillary border presents a well-defined golden line nearly half a millimetre wide,

<sup>1</sup> MELTZER and AUER: This journal, 1904, xi, p. 28.

while the rest of the fairly broad iris looks as if it were more or less thickly covered with gold dust. The normal pupil reacts to light slowly but distinctly.

The effect of adrenalin upon the pupil, whether applied by subcutaneous injection or instillation, is to increase all its diameters, but primarily and most extensively the vertical one, causing the pupil to become almost entirely round. The notch becomes nearly obliterated.

We shall illustrate our observations by a few abbreviated protocols of experiments.

#### INJECTIONS INTO THE LYMPH SACS.

*Experiment 1. May 13, 1904.* — Large frog; pupils moderately dilated.

5.00 P. M. Injected into dorsal sac 0.1 c.c. adrenalin (1 : 1000).

5.08. Pupils a trifle dilated, anterior pole rounder.

5.15. Pupils wider, rounder. Upper part of iris very narrow; pupils do not react to light. (Left pupil not as large as right — small corneal infiltration.)

5.30 and 8.00. Condition the same.

*May 14.* — 8.30 A. M. Pupils smaller than before, but still somewhat dilated.

2.00 P. M. Pupils almost same as before. At no time was there any prostration of the animal. A subcutaneous injection of a small dose of adrenalin, which had otherwise no effect upon the frog, caused in eight minutes a dilatation of the pupils which lasted about twenty-one hours.

*Experiment 2. May 13, 1904.* — Small frog, pupils moderately dilated.

5.02 P. M. Injected into dorsal sac 0.05 c.c. adrenalin.

5.10. Pupils widely dilated, almost round; do not react to light.

5.30. Condition same.

5.45. Pupils a trifle smaller again, not quite as round as before.

8.30. Pupils about same as before injection. Such a small dose as 0.05 c.c. brought out a distinct dilatation of the pupils in eight minutes; but here the dilatation began to disappear already in forty-five minutes, and was gone entirely in a few hours.

*Experiment 3. May 16, 1904.* — Large frog; pupils equal.

9.06 A. M. Injected into dorsal sac 0.3 c.c. adrenalin.

9.14. Beginning dilatation very definite in both diameters.

9.30. Pupils dilated to about  $1\frac{1}{2}$  times their original size; react very slightly to light.

9.47. Second injection of 0.3 c.c. into ventral sac.

10.01. Pupils wider than before; animal quiet.

- 10.30. Pupils a little narrower again. Animal prostrated.  
4.25 P. M. Pupils wide as last note.  
8.30 P. M. Pupils still wide, but less than before. Observation discontinued.

*Experiment 4. May 16, 1904.* — Medium-sized frog; pupils equal, horizontal diameter  $5\frac{1}{2}$  mm., vertical 4 mm.

- 3.50 P. M. Injected 0.2 c.c. adrenalin into dorsal sac.  
3.55. Both pupils, horizontal diameter as before, vertical,  $4\frac{1}{2}$ .  
4.06. Horizontal,  $5\frac{3}{4}$ ; vertical, 5 mm.  
5.00 and 8.20. No change.  
6.00 P. M., May 17. Right pupil,  $5 \times 4\frac{1}{2}$ ; left,  $5 \times 4$ .

*Experiment 5. May 16, 1904.* — Medium-sized frog; pupils equal,  $4 \times 3$ .

- 4.01 P. M. Injected 0.5 c.c. into dorsal sac.  
4.04.  $4\frac{3}{4} \times 4$ ; both pupils equal.  
4.26.  $4\frac{1}{2} \times 4\frac{1}{4}$ ; pupils equal.  
5.10. Right,  $4\frac{1}{2} \times 4$ ; left,  $4\frac{1}{2} \times 4\frac{1}{2}$ .  
8.20. Both pupils,  $5 \times 4$  mm.  
6 P. M., May 17. Both pupils,  $4\frac{1}{2} \times 3\frac{3}{4}$ . Observation discontinued.

Many other experiments have given similar results.

A small dose of 0.1 c.c. of adrenalin will generally have the same effect upon the pupils of a frog as a dose of 0.5 c.c. and more. While, however, the latter dose will invariably cause a pronounced prostration of the animal, a dose of 0.1 c.c. has no other perceptible effect upon the frog than the dilatation of the pupils. A smaller dose than 0.1 c.c. might also have some effect upon the pupils, but the effect apparently passes off much sooner. The dilating effect set in three to eight minutes after the injection. The first effect was always observed to take place in the vertical diameter. Sooner or later, however, the horizontal diameter also invariably became longer. Proportionately the vertical diameter was almost always the more affected one; hence, the change to the round shape.

The reaction to light was almost always absent.

The dilatation was rarely a maximum one; there remained all around a perceptible portion of the iris. The golden pupillary line of the iris became in all cases distinctly narrower. The maximum effect lasted in some cases many hours, in others only a comparatively short time—in some cases even less than an hour. The reduction, however, was usually only a trifle, most of the dilatation continued for many hours, and in some cases a part of it was unmis-

takably present twenty-four hours and more after the subcutaneous injection.

#### THE EFFECT OF INSTILLATION.

Instillation of a few drops of adrenalin into the conjunctival sac of one eye had invariably a dilating effect upon the pupil of that eye. Usually the closure of the (lower) lid rapidly wipes away anything which is applied to the eye. Most of the instillations were carried out by pushing a fine pipette between the bulbus and the lid. Two to three drops of adrenalin were sufficient to bring out in three to seven minutes a dilatation of the pupil which was as strong and lasted as long as when produced by subcutaneous injection. However, to meet the objection that by pushing the pipette into the conjunctival sac some of the epithelium might have become abraded, in some cases the adrenalin was simply dropped on the eyeball without touching it. In these experiments also the pupil became dilated, but usually a longer interval passed before the dilatation set in. In some cases, especially when more drops were used, the other pupil also became slightly dilated, due apparently to an absorption of some adrenalin into the circulation. In most cases, pains were taken to wipe away any adrenalin flowing over the surface adjoining the eye, — in order to avoid absorption through the skin or through the nasal orifices. The following are a few illustrations of experiments:

*Experiment 6. May 16, 1904.* — Medium-sized frog; pupils equal,  $3\frac{1}{2} \times 2\frac{3}{4}$  mm.

- 4.12 P. M. Instilled into right sac one drop.
- 4.15. Another drop.
- 4.16. Right pupil,  $3\frac{1}{2} \times 3$ .
- 4.31. Right,  $4 \times 3\frac{3}{4}$ ; left,  $3\frac{1}{2} \times 3$ .
- 5.01. Right,  $4 \times 4$ ; left,  $3\frac{1}{2} \times 3\frac{1}{4}$ .
- 8.30. Right,  $4 \times 3\frac{3}{4}$ .

*Experiment 7. May 20, 1904.* — Medium-sized frog; pupils equal,  $3\frac{1}{2} \times 3$  mm.

- 6.08 P. M. Instilled one drop into right eye.
- 6.09. Instilled another drop.
- 6.11. Right pupil,  $3\frac{3}{4} \times 3\frac{1}{4}$ .
- 6.37. Right,  $4 \times 3\frac{3}{4}$ ; left,  $3\frac{1}{2} \times 3$ .
- 7.09. Right,  $4 \times 4$ ; left,  $3\frac{1}{2} \times 3$ .
- 10.25. Right,  $4 \times 3\frac{3}{4}$ ; left,  $3\frac{1}{2} \times 3$ .
- 2.30 A. M., May 21. Right,  $3\frac{3}{4} \times 3\frac{1}{2}$ ; left,  $3\frac{1}{2} \times 3$ .
- 8.00. Both pupils,  $3\frac{1}{2} \times 3$ .

*Experiment 8. May 23, 1904.* — Small frog; pupils equal,  $3\frac{1}{2} \times 2\frac{3}{4}$ .

2.39 P. M. Instilled two drops into right sac.

2.41. Instilled one drop into right sac.

2.43. Right pupil,  $4 \times 3\frac{3}{4}$ .

2.54. Right,  $4 \times 3\frac{3}{4}$ ; left,  $3\frac{1}{2} \times 2\frac{3}{4}$ .

2.57. Injected into dorsal sac 0.3 c.c. adrenalin.

2.59. Right,  $4 \times 3\frac{3}{4}$ ; left,  $3\frac{1}{2} \times 3\frac{1}{2}$ .

4.05. Right and left,  $4 \times 3\frac{3}{4}$ .

8.15. Right and left,  $4 \times 3\frac{1}{2}$ .

8.50 A. M., May 24. Right and left,  $3\frac{1}{2} \times 3$ .

10.45. Right and left,  $3\frac{1}{2} \times 2\frac{3}{4}$ .

In this last experiment the subsequent subcutaneous injection did not increase the dilatation of the right pupil brought on by instillation, nor did the degree of the dilatation of the left pupil brought on by the injection differ from that of the right pupil. There was also no difference between the two pupils in the duration of the dilatation.

In another series of experiments the effect of adrenalin upon the pupils was studied after the elimination of the central nervous system. We shall record the results briefly without illustrating them by experiments.

1. The cord was severed just below the medulla oblongata. It often occurred that after the operation the pupils became very small. In such animals the effect of injection as well as of instillation was indeed striking. From small slitlike apertures the pupils became large and round, as large and larger than in normal animals.

2. When the head was cut off at a line connecting the anterior borders of the ear drums, instillation into the conjunctival sac brought out a prompt dilatation of the pupil.

3. Finally, in carefully excised eyes, dropping of adrenalin upon the corneal surface, brought on promptly a dilatation of the pupil which lasted many hours. The effect was still distinct, even when the application of the adrenalin took place about five hours after excision, if the eyes were kept moist. When applied seven hours after excision, the effect was slight and brief.

The most interesting fact of our observations is the striking difference between frogs and mammals. While in normal frogs a subcutaneous injection or instillation of suprarenal extract has an unmistakable effect upon the pupils, such an effect can be obtained in mammals by these methods of administration, only some time after the removal of the superior cervical ganglion, and even then the

effect is by far not so prolonged as seen in frogs. We shall not enter, for the present at least, into a discussion of the possible nature of this difference.

It is noteworthy that the dose which is capable of bringing out a maximum effect upon the pupils in frogs, is only a small fraction of that which is necessary to produce a general effect. A further increase of the dose neither increases the dilatation of the pupil nor does it essentially prolong the effect.

The length of the effect is especially noteworthy. The chief knowledge of the effects of the suprarenal extract is derived from intravenous injections. By this method of application the effect upon the blood-pressure lasts only five or six minutes, and the effect upon the pupil only about one minute. We have shown that the cutting of the sympathetic nerve prolongs the effect upon the blood-vessels (ears),<sup>1</sup> and the removal of the superior cervical ganglion prolongs the effect upon the pupil. But even in these cases the prolongation rarely exceeds two or three hours. In the observations on normal frogs, however, the pupils remain dilated twenty-four hours and longer, and this even in normal frogs with good circulation.

Finally we wish to call attention to the fact that the frog's eye, excised or *in situ*, might prove to be a better reagent than the blood-pressure to demonstrate the efficiency of a suprarenal preparation.

<sup>1</sup> MELTZER and AUER: This journal, 1903, ix, p. 252.



# THE NATURE OF CHEMICAL AND ELECTRICAL STIMULATION. I.—THE PHYSIOLOGICAL ACTION OF AN ION DEPENDS UPON ITS ELECTRICAL STATE AND ITS ELECTRICAL STABILITY.<sup>1</sup>

By A. P. MATHEWS.

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IT is now well established that the chemical properties of an element vary with its electrical state. Iodine in sodium iodide has different chemical properties from iodine itself; nascent (negative) hydrogen reduces, while positive hydrogen oxidizes. The same difference exists between ferric iron, which oxidizes, and ferrous iron, which reduces.

The fact that the chemical nature of any substance depends on its electrical condition suggests at once that its physiological or pharmacological action will also depend on the same factor, for the living protoplasm is but the seat of numerous chemical reactions which are modified by the salts or drugs.

The evidence of the truth of this conclusion is already abundant. We have long known that ferric and ferrous salts act differently. For example, ferric salts are poisonous to *Fundulus* embryos in  $\frac{1}{120000}$  concentration, while ferrous salts require a concentration of  $\frac{1}{200}$  to kill in the same time.<sup>2</sup> The difference between these salts is in the electrical state of the iron. It has long been apparent that negative and positive chlorine must have different actions, for the chlorine in sodium chloride stimulates motor nerves, whereas gaseous chlorine anæsthetizes them. It is known also, that the albuminate of mercury is less poisonous than mercuric chloride, which contains so many positive mercury atoms. It is clear from these facts and the observations

<sup>1</sup> A preliminary report of a portion of the results in this paper was published in *Science*, 1902, xv, p. 492; *Ibid.*, 1903, xvii, p. 729; *Yale medical journal*, June, 1903, p. 1.

<sup>2</sup> MATHEWS: This journal, 1904, xi, p. 298.

of Kahlenberg and True, Krönig and Paul, Pauli, Cole, Loeb,<sup>1</sup> and others, that the physiological action of a substance depends on its particular electrical condition. The author has shown that the poisonous power of salts is a function of their electrolytic decomposition tensions, and McGuigan<sup>2</sup> has shown that their anti-fermentative powers depend on this also.

The present paper contains further evidence in support of the truth of the foregoing propositions, and in addition thereto sets forth that not only do the atoms change their actions with their electrical states, but *that they act by means of their electrical condition or their electrical charges ; and that positively and negatively charged ions have opposite actions*. All ions of the same sign act alike ; but they differ in the degree of their action, because the ease with which they change their electrical condition, or give up their charges, varies. This latter factor is measured by the solution tension.<sup>3</sup>

While it is not necessary to adopt the electron hypothesis to explain these facts, I shall, for convenience and clearness, express them in terms of that hypothesis, and speak of a charge or electron being taken from or added to an atom. If this particular hypothesis be rejected, the essential facts demonstrating the importance of the electrical condition of the atom will remain unaltered.

The final conclusion is simply stated as follows : The ions of the inorganic and organic salts in protoplasm are nothing else than very minute, freely moving electrodes. They are hence constantly stimulating the protoplasm by means of their electrical charges. The same laws hold for the action of these ions as for the action of electrodes, *i. e.*, the oppositely charged ions have opposite actions ; the positive ion depresses most forms of protoplasm, the negative stimulates.<sup>4</sup> The power of any ion depends on the potential of the ion, which is the reciprocal of its solution tension, and upon the factor corresponding to the density of the current, *i. e.*, the concentration of the ion. Just as in an electrode the chemical composition of the electrode is

<sup>1</sup> KAHLÉNBERG and TRUE : Botanical Gazette, 1896, xxii, p. 91 ; KRÖNIG and PAUL : Zeitschrift für Hygiene, 1897, xxv, p. 1 ; PAULI : Beiträge zur chemischen Physiologie, 1902, iii, p. 225 ; LOEB : This journal, 1902, vi, p. 471 ; COLE : Journal of physiology, 1903, xxx, p. 202, 282.

<sup>2</sup> MCGUIGAN : This journal, 1904, x, p. 444.

<sup>3</sup> This suggestion concerning the physiological action of ions and their solution tension was first made by Prof. J. STIEGLITZ.

<sup>4</sup> From other reasoning, STRONG, Journal of physiology, 1900, xxv, p. 427, has also suggested that the anions stimulated.

of little importance, so the chemical composition of the ion is of little importance, compared with the importance of its electrical condition.

This conclusion clears up many of the actions of inorganic salts on protoplasm, and indirectly also it explains some of the phenomena of electrical stimulation, since such stimulation is due simply to the accumulation of negative or positive ions in different places in the tissue, or, in other words, to differences in concentration of the ions, as suggested by Nernst.<sup>1</sup>

This conclusion is a result or extension of the work of many investigators, being foreshadowed in the work of Kahlenberg and True and Krönig and Paul, when they demonstrated that substances acted by their ions. It was not, however, recognized by these investigators. Loeb<sup>2</sup> first suggested that the valence or possibly the electrical charge might determine the action of ions, at least in part. This suggestion was made tentatively, and the facts Loeb adduced were not sufficient to show its truth. In a later paper<sup>3</sup> Loeb unfortunately withdrew this hypothesis. Loeb did not apparently recognize that this hypothesis necessitated the conclusion of the opposite action of oppositely charged ions.

I have studied the action of salts and non-electrolytes on the sciatic nerve of the frog. The whole sciatic nerve and the gastrocnemius muscle were prepared and suspended by the femur in a moist chamber, the muscle attached to a lever writing on a drum, and the nerve immersed in a few cubic centimetres of the solution to be tested. About sixteen hundred experiments have been tried at different seasons of the year, in order to throw out seasonal and individual variations, which are known to be considerable. I have used the leopard frog, *Rana pipiens* (Schreber) almost exclusively. The records of all experiments were preserved. The concentration of the weakest stimulating solution has been determined more accurately for some salts than for others. Where it is doubtful, I have put a question mark.

There have been numerous studies of the chemical stimulation of the motor nerve by solutions of electrolytes and non-electrolytes, which have been well summarized up to 1893 by Grützner.<sup>4</sup> As a result of

<sup>1</sup> NERNST: *Zur Theorie der elektrischen Reizung*, Nachrichten von der Königliche Gesellschaft der Wissenschaften zu Göttingen. Math.-Physikal. Klasse. 1899, p. 104.

<sup>2</sup> LOEB: *Archiv für die gesammte Physiologie*, 1901, lxxxviii, p. 68; *This journal*, 1902, vi, p. 411.

<sup>3</sup> LOEB: *Archiv für die gesammte Physiologie*, 1902, xci, p. 255.

<sup>4</sup> GRÜTZNER: *Archiv für die gesammte Physiologie*, 1893, lii, p. 83.

this work physiologists are of the opinion that most substances stimulate by dehydrating the nerve. Grützner and several more recent observers, however, have pointed out that some salts stimulate in solutions too weak to dehydrate. These salts evidently stimulate in some other manner than by dehydration, but how they stimulate has not been discovered. Nor are there accurate determinations for the greater number of salts of the minimum strength of solution which will stimulate. In the absence of this information it was impossible to form any probable opinion concerning the manner in which salts stimulated. At the beginning of the investigation Loeb's<sup>1</sup> paper appeared, in which it was suggested that specific ions, *i. e.*, sodium, hydrogen, or hydroxyl ions, stimulated muscle to contract, but, so far as I know, no suggestions have been made that similar relations hold for the nerve, although that such was the case was indicated by the observations of Grützner, Limbourg,<sup>2</sup> and others, who found that certain sodium salts would stimulate in dilute solutions. My first experiments were undertaken to find out the minimum strength of solution of different salts and non-electrolytes which would stimulate, in order to see whether stimulation was always due to osmosis.

#### I. NON-ELECTROLYTES.

In Table I, Column 2 gives the concentration of the most dilute stimulating solution. It will be understood that stronger solutions stimulated, unless the contrary is stated.

**Summary: Non-electrolytes.** — With the exception of alcohol and acetone, all non-electrolytes investigated require a concentration of at least one-half molecular to stimulate. Such a solution has an osmotic pressure of twelve atmospheres, which is about twice that of the nerve. As they do not stimulate in dilute solution, we may conclude that these non-electrolytes stimulate in virtue of their concentration, either directly by the extraction of water, or, what is less probable (see p. 472), by increasing indirectly the concentration of salts in the outer part of the nerve, and thus causing a concentration chain. No non-electrolyte was found to possess in isotonic solutions a stimulating action on the nerve, but it is not impossible that such substances will be found, particularly as there are probably such substances stimulating the nerve cell. Strong reducing agents should stimulate.

<sup>1</sup> LOEB: Fick's Festschrift, Braunschweig, 1900.

<sup>2</sup> LIMBOURG: Archiv für die gesammte Physiologie, 1887, xli, p. 303.

## II. ELECTROLYTES.

**Sodium salts.** — Table III shows a series of experiments comparing the action of the fluoride and chloride on the different nerves of the same frog, and a similar comparison of the oxalate and thiosulphate. These experiments illustrate the superior stimulating power of the fluoride and oxalate.

**Summary and conclusions.** — Table IV shows the weakest solutions of the sodium salts which will stimulate, grouped by the valence of the anion.

These results may be summarized as follows: (1) All sodium salts examined will stimulate if their solutions have an osmotic pressure of twelve to thirteen atmospheres. (2) *With the exception of the iodate,*

TABLE I.  
RESULTS FOR MARCH AND APRIL FROGS.

Substance.	Weakest stimulating solution.	Approximate osmotic pressure in atmospheres.	Latent period for weakest solution.	Remarks.
			minutes	
Cane-sugar	$\frac{m}{2}$	12	18	Prolonged, tetanic contractions.
Glucose . .	$\frac{m}{2}$	12	15	Prolonged, tetanic contractions.
Levulose . .	$m\frac{1}{2} - \frac{3}{4}$	12	15	Small tetanus.
Glycerine . .	$\frac{m}{2}$	12	13	Long tetanus.
Urea . . .	$\frac{m}{2}$	12	13	Very long tetanus.

*carbonate, bicarbonate, bisulphate, bichromate, chromate, valerianate, and salicylate, all will stimulate in solutions which have the same osmotic pressure as the nerve, or a weaker pressure.* It is clear from this, that while all sodium salts may stimulate by osmosis, most of them are able to stimulate in some other way than by the extraction of water, or by means of concentration.

Before proceeding to a discussion of the way in which these salts stimulate, it is first necessary to show that they produce their effect by action on the nerve and not on the muscle. The long latent period required before the stimulation began with sodium chloride and many of the other salts led to the suspicion that they were in reality diffusing down the nerve into the muscle and were producing their effect in this way. Loeb had already shown that sodium salts

TABLE II.  
SODIUM SALTS.

I. Salt.	II. Concentra- tions stimulat- ing. In terms of molecular solution.	III. Concentra- tions not stimulating.	IV. Limiting stimu- lating concen- trations.	V. Latent period in min- utes for $\frac{m}{8}$ solu- tions.	VI. Character of contractions.
Chloride . . . . .	2, 1, $\frac{4}{5}$ , $\frac{1}{3}$ , $\frac{1}{4}$ , $\frac{1}{5}$ , $\frac{1}{7}$ , $\frac{1}{8}$ , $\frac{1}{5}$ , $\frac{1}{10}$ , $\frac{1}{11}$ .	$\frac{1}{12}$ , $\frac{1}{15}$ , $\frac{1}{20}$	$\frac{m}{11}$	160-240	Tetanus and rhythmic. 3 hours.
Bromide . . . . .	1, $\frac{1}{2}$ , $\frac{1}{3}$ , $\frac{1}{8}$	$\frac{1}{10}$ , $\frac{1}{12}$	$\frac{m}{16}$	180-240	Less than NaCl.
Iodide . . . . .	$\frac{1}{2}$ , $\frac{1}{3}$ , $\frac{1}{8}$ , $\frac{1}{12}$	$\frac{1}{15}$ , $\frac{1}{20}$	$\frac{m}{12}$	16(?) - 120	Like NaCl.
Nitrate . . . . .	$\frac{1}{2}$ , $\frac{1}{3}$ , $\frac{1}{4}$ , $\frac{1}{5}$ , $\frac{1}{6}$ , $\frac{1}{8}$	....	$\frac{m}{11}$ (?)	160-240	Like NaCl.
Fluoride . . . . .	$\frac{1}{2}$ , $\frac{1}{7}$ , $\frac{1}{8}$ , $\frac{1}{10}$ , $\frac{1}{12}$	$\frac{1}{16}$ , $\frac{1}{20}$	$\frac{m}{12}$	20	High tetanus.
Acetate . . . . .	1, $\frac{1}{3}$ , $\frac{1}{4}$ , $\frac{1}{5}$ , $\frac{1}{6}$ , $\frac{1}{8}$	$\frac{1}{20}$	$\frac{m}{11}$	160	Long continued rhythmic.
Chlorate . . . . .	$\frac{1}{2}$ , $\frac{1}{8}$	$\frac{1}{15}$	$\frac{m}{11}$ (?)	60-80	Very prolonged rhythmic.
Bromate . . . . .	$\frac{1}{2}$ , $\frac{1}{3}$ , $\frac{1}{6}$ , $\frac{1}{8}$	....	$< \frac{m}{8}$	30-120	Long rhythmic.
Iodate . . . . .	$\frac{1}{4}$	$\frac{1}{5}$ , $\frac{1}{8}$ , $\frac{1}{10}$	$\frac{m}{4}$		
Permanganate . . . . .	$\frac{1}{3}$ , $\frac{1}{5}$ , $\frac{1}{9}$ , $\frac{1}{10}$ , $\frac{1}{12}$	$\frac{1}{12}$ , $\frac{1}{13}$	$\frac{m}{12}$	2	Short, high tetanus.
Hydrate . . . . .	1, $\frac{1}{5}$ , $\frac{1}{10}$ , $\frac{1}{15}$ , $\frac{1}{20}$	$\frac{1}{25}$ , $\frac{1}{30}$	$\frac{m}{20}$	1	Short, high tetanus.
Formate . . . . .	$\frac{1}{4}$ , $\frac{1}{10}$ , $\frac{1}{12}$	$\frac{1}{16}$ , $\frac{1}{20}$	$\frac{m}{12}$	25	Long rhythmic.
Sulphocyanide . . . . .	$\frac{1}{3}$ , $\frac{1}{5}$ , $\frac{1}{8}$ , $\frac{1}{10}$	$\frac{1}{12}$ , $\frac{1}{18}$	$\frac{m}{16}$	160-240	Like NaCl.
Salicylate . . . . .	....	$\frac{1}{7}$ , $\frac{1}{8}$ , $\frac{1}{10}$ , $\frac{1}{20}$			
Valerianate . . . . .	....	$\frac{1}{8}$			
Butyrate . . . . .	1, $\frac{1}{10}$	....	$< \frac{m}{10}$	30	Stronger than NaCl.
Chromate . . . . .	$\frac{1}{2}$ , $\frac{1}{3}$	$\frac{1}{4}$ , $\frac{1}{5}$	$\frac{m}{8}$		
Nitrite . . . . .	1, $\frac{1}{11}$	$\frac{1}{8}$	$\frac{m}{11}$	160-240	Like NaCl.
Oxalate . . . . .	$\frac{1}{2}$ , $\frac{1}{4}$ , $\frac{1}{6}$ , $\frac{1}{8}$ , $\frac{1}{15}$ , $\frac{1}{16}$ , $\frac{1}{20}$ , $\frac{1}{30}$	$\frac{1}{40}$ , $\frac{1}{80}$ , $\frac{1}{10}$ , $\frac{1}{100}$	$\frac{m}{35}$	10	High tetanus.
Thiosulphate . . . . .	$\frac{1}{2}$ , $\frac{1}{8}$ , $\frac{1}{15}$ , $\frac{1}{32}$	$\frac{1}{35}$	$\frac{m}{32}$	45	Low tetanus.
Sulphite . . . . .	$\frac{1}{2}$ , $\frac{1}{4}$ , $\frac{1}{8}$ , $\frac{1}{15}$ , $\frac{1}{15}$ , $\frac{1}{32}$ , $\frac{1}{35}$	$\frac{1}{40}$	$\frac{m}{36}$	45	Low tetanus.
Sulphate . . . . .	$\frac{1}{2}$ , $\frac{1}{8}$ , $\frac{1}{15}$ , $\frac{1}{35}$ , $\frac{1}{40}$ , $\frac{1}{75}$ , $\frac{1}{120}$ , $\frac{1}{24}$ , $\frac{1}{25}$ , $\frac{1}{32}$	$\frac{1}{40}$	$\frac{m}{32}$	20	Prolonged low tetanus.
Tartrate . . . . .	$\frac{1}{3}$ , $\frac{1}{4}$ , $\frac{1}{5}$ , $\frac{1}{6}$ , $\frac{1}{10}$ , $\frac{1}{16}$ , $\frac{1}{20}$ , $\frac{1}{30}$ , $\frac{1}{35}$ , $\frac{1}{40}$	$\frac{1}{45}$	$\frac{m}{40}$	5	High tetanus.
Bicarbonate . . . . .	$\frac{1}{2}$ , $\frac{1}{3}$	$\frac{1}{4}$ , $\frac{1}{5}$ , $\frac{1}{9}$ , $\frac{1}{15}$	$\frac{m}{3}$		

TABLE II—continued.

I. Salt.	II. Concentra- tions stimulat- ing. In terms of molecular solution.	III. Concentra- tions not stimulating.	IV. Limiting stimu- lating concen- trations.	V. Latent period in min- utes for $\frac{n}{8}$ solu- tions.	VI. Character of contractions.
Carbonate . . . . .	$\frac{1}{2}, \frac{1}{4}, \frac{1}{6}, \frac{1}{8}, \frac{1}{10}$	$\frac{1}{2}, \frac{1}{4}, \frac{1}{6}, \frac{1}{8}, \frac{1}{10}, \frac{1}{12}, \frac{1}{14}, \frac{1}{16}, \frac{1}{18}, \frac{1}{20}, \frac{1}{24}, \frac{1}{32}, \frac{1}{40}, \frac{1}{48}, \frac{1}{60}$	$\frac{m}{7}(?)$	3(?)	Tetanus.
Borax . . . . .	$\frac{1}{3}, \frac{1}{6}, \frac{1}{9}, \frac{1}{10}, \frac{1}{11}, \frac{1}{12}$	$\frac{1}{15}$	$< \frac{m}{11}$	120	Rhythmic.
Acid phosphate . . . . .	$\frac{1}{2}, \frac{1}{4}, \frac{1}{6}, \frac{1}{8}, \frac{1}{10}, \frac{1}{12}, \frac{1}{15}$	$\frac{1}{3}, \frac{1}{9}$	$\frac{m}{7}$		
Di-sodium phosphate . . . . .	$\frac{1}{3}, \frac{1}{5}, \frac{1}{6}, \frac{1}{8}, \frac{1}{10}, \frac{1}{12}, \frac{1}{15}, \frac{1}{18}, \frac{1}{20}, \frac{1}{24}, \frac{1}{30}, \frac{1}{40}, \frac{1}{60}$	$\frac{1}{15}$	$\frac{m}{4}$	38	Long, low tetanus.
Di-sodium arsenate . . . . .	$\frac{1}{3}, \frac{1}{4}, \frac{1}{6}, \frac{1}{8}, \frac{1}{10}, \frac{1}{12}, \frac{1}{15}, \frac{1}{20}$	$\frac{1}{15}$	$< \frac{m}{10}$	3	Long tetanus.
Di-sodium arsenite . . . . .	$\frac{1}{30}, \frac{1}{60}$	....	$\frac{m}{6}$	....	Tetanus.
Succinate . . . . .	$\frac{1}{3}, \frac{1}{6}$	$\frac{1}{15}$	$\frac{m}{16}$	120	
Bichromate . . . . .	$\frac{1}{4}, \frac{1}{2}$	$\frac{1}{4}, \frac{1}{6}, \frac{1}{8}, \frac{1}{10}$	$\frac{m}{4}$		
Ferricyanide . . . . .	$\frac{1}{30}, \frac{1}{60}, \frac{1}{120}$	$\frac{1}{60}, \frac{1}{120}$	$\frac{m}{10}$	27(?)	Long, high tetanus.
Tri-sodium citrate . . . . .	$\frac{1}{30}, \frac{1}{40}, \frac{1}{60}, \frac{1}{80}, \frac{1}{120}, \frac{1}{150}, \frac{1}{180}, \frac{1}{240}, \frac{1}{300}, \frac{1}{360}, \frac{1}{420}, \frac{1}{600}$	$\frac{1}{60}$	$\frac{m}{30}-$	2	High tetanus.

TABLE III.

Salt.	Concentration.	Latent period.	Contractions.
		minutes	
1. { NaCl	$\frac{1}{10}$	120	Rhythmic.
{ NaFl	$\frac{1}{10}$	20	Tetanic.
2. { NaCl	$\frac{1}{10}$	240	Individual.
{ NaFl	$\frac{1}{10}$	37	Tetanic.
3. { NaCl	$\frac{1}{12}$	240	Individual.
{ NaFl	$\frac{1}{12}$	35	Tetanus, low.
4. { NaCl	$\frac{1}{15}$	$\infty$	0
{ NaFl	$\frac{1}{15}$	$\infty$	0
5. { Na <sub>2</sub> C <sub>2</sub> O <sub>4</sub>	$\frac{m}{20}$	18	High tetanus.
{ Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>	$\frac{m}{20}$	60	" "
6. { Na <sub>2</sub> C <sub>2</sub> O <sub>4</sub>	"	15	High tetanus.
{ Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>	"	60	" "

would cause rhythmic contractions of the muscle. The following experiment showed the action was on the nerve:

If the nerves of curarized frogs were placed in solutions of stimulating salts, the muscles did not contract. Control experiments showed that if the curarized muscle was placed directly in the salt solutions fibrillar contractions occurred. The experiment was tried of etherizing

TABLE IV.

MONO-VALENT SALTS.		BI-VALENT SALTS.		TRI-VALENT SALTS.	
Salt.	Concentration.	Salt.	Concentration.	Salt.	Concentration.
NaCl	$\frac{m}{12}$	$Na_2CO_3$	$\frac{m}{8} (?)$ or $\frac{m}{6}$	$Na_2HPO_4$	$\frac{m}{35} (?)$
NaBr	$\frac{m}{12}$	$NaHCO_3$	$\frac{m}{5}$	$NaH_2PO_4$	$\frac{m}{6}$
NaI	$\frac{m}{13}$	$Na_2SO_4$	$\frac{m}{32}$	$Na_3$ citrate	$\frac{m}{50}$
$NaClO_3$	$\frac{m}{12}$	$Na_2SO_3$	$\frac{m}{35}$	$Na_2HAsO_4$	$\frac{m}{12} (?)$
$NaBrO_3$	$\frac{m}{12}$	$Na_2B_4O_7$	$\frac{m}{15} (?)$	$Na_2HASO_3$	$\frac{m}{50}$
$NaIO_3$	$\frac{m}{4}$	$NaHSO_4$	$\frac{m}{6}$	$Na_4FeCn_6$	$\frac{m}{50}$
$NaH_3C_2O_2$	$\frac{m}{12}$	$Na_2$ tartrate	$\frac{m}{15}$		
$NaHCO_2$	$\frac{m}{12}$	$NaK$ tartrate	$\frac{m}{5} (?)$		
NaCNS	$\frac{m}{7} (?)$	$Na_2C_2O_4$	$\frac{m}{12}$		
$Na valerianate$	$> \frac{m}{4}$	$Na_2S_2O_3$	$\frac{m}{12}$		
Na butyrate	$\frac{m}{12}$	$Na_2Cr_2O_7$	$\frac{m}{5}$		
$NaNO_3$	$\frac{m}{12}$				
$NaNO_2$	$\frac{m}{12}$				
$NaCrO_4$	$\frac{m}{12}$				
$NaMnO_4$	$\frac{m}{12}$				
NaOH	$\frac{m}{20}$				

the nerve between the solution and the muscle by passing the nerve through a small rubber tube which lay close to the muscle. The end of the nerve was put in the solution. When the muscle began to contract, ether vapor was drawn through the tube, thus etherizing a small piece of the nerve close to the muscle. As soon as the nerve was etherized, contractions ceased. On drawing air through the tube,



they began again. Inasmuch as the ether would not in all likelihood stop the diffusion of the sodium salts down the nerve, were such a diffusion taking place, it is clear that the impulses arose from the action of the salt on the nerve and not on the muscle. Another proof is obtained by cutting the end of the nerve while the muscle is contracting. If this is done, the contractions cease after a short tetanus following the cut.

**Potassium salts.** *Potassium chloride.*—The results obtained with potassium chloride are somewhat inconstant. As a rule, solutions of  $m$ ,  $\frac{m}{2}$ ,  $\frac{m}{3}$ , or  $\frac{m}{4}$  will stimulate. But this is not always the case. Solutions weaker than this never stimulate, but instead very rapidly depress the nerve. The contractions obtained in the stronger solutions may be tetanic, high, and last but from five to fifteen minutes. The latent period is shorter than with the corresponding sodium chloride solutions. Potassium chloride, in solutions isotonic with the nerve, will not stimulate. The stimulation in stronger solutions is due to osmosis, as the weakest stimulating solution has an osmotic pressure of about thirteen atmospheres. The fact that in these strong solutions the potassium chloride solutions will stimulate motor nerves confirms the observations of Limbourg,<sup>1</sup> and shows that Grützner's statement<sup>2</sup> that this salt will not stimulate motor nerves, requires modification. This fact renders doubtful the distinction between sensory and motor nerves, which was supposed to be shown by the stimulation of the sensory nerve by this salt. It is, however, true that in solutions of the same osmotic pressure as the nerve, this salt never stimulates, and in this respect has a totally different action from sodium chloride. I have tried a number of experiments with the sensory end of the sciatic nerve to see if, as Grützner states, potassium stimulates it more strongly than sodium, but without any satisfactory result. The reflex response, unless one uses strong solutions which may act by osmosis, is very uncertain.

The nerve, after losing its irritability in potassium chloride, may be recovered if placed in  $\frac{m}{8}$  sodium chloride solution. This is shown in a later experiment.

The results with the other potassium salts may be seen in the following table:

<sup>1</sup> LIMBOURG: *Archiv für die gesammte Physiologie*, 1887, xli, p. 303.

<sup>2</sup> GRÜTZNER: *Loc. cit.*

TABLE V.  
POTASSIUM SALTS.

Salt.	Weakest stimulating solution.	Remarks.
KCl	-	Rapid depression after stimulation.
KBr	$\frac{1}{4}$	Rapid depression after stimulation.
KI	-	Rapid depression after stimulation.
KNO <sub>3</sub>	$\frac{m}{5}$	Rapid depression after stimulation.
KClO <sub>3</sub>	$\frac{m}{4}$	Rapid depression after stimulation.
KMnO <sub>4</sub>	$\frac{m}{12}$	Short tetanus.
KOH	$\frac{m}{2.6}$	Short tetanus.
K <sub>2</sub> C <sub>2</sub> O <sub>4</sub>	$\frac{m}{1.6}$	Short tetanus.
K <sub>3</sub> citrate	$\frac{m}{2.6}$	Short tetanus.
KCy	$\frac{m}{5}$	Tetanus and depression.
KFl	$\frac{m}{5}$	Tetanus and depression.
K <sub>2</sub> SO <sub>4</sub>	$\frac{m}{5}$	Tetanus and depression.
KHCO <sub>3</sub>	$\frac{m}{4}$	Tetanus and depression.
KHSO <sub>4</sub>	$\frac{m}{4}$	Very rapid depression.
KH <sub>2</sub> PO <sub>4</sub>	$> \frac{m}{1.6}$	No contractions in $\frac{m}{1.6}$ or weaker. Stronger not tried.
K <sub>4</sub> FeCy <sub>6</sub>	$\frac{m}{1.6}$	In a few cases only.
K <sub>3</sub> FeCy <sub>6</sub>	$\frac{m}{1.6}$	In a few cases only.
K <sub>2</sub> tartrate	$\frac{m}{1.2}$	Strong, short tetanus.

**Summary: Potassium salts.**—The potassium salts will generally stimulate if their solutions have an osmotic pressure of thirteen atmospheres or over. When they stimulate, therefore, most of them do so by osmosis or concentration. *Some of them, however, i. e., the hydrate, tartrate, citrate, and possibly the ferrocyanide, stimulate in solutions too weak to dehydrate the nerve.* This fact demonstrates that stimulation in solutions isotonic with the nerve is not a peculiar function of sodium salts.

**Summary: Ammonium salts.**—The ammonium salts resemble potassium in that they rapidly destroy irritability. They are not so powerful as potassium in this particular. The citrate stimulated in

the solutions too weak to dehydrate the nerve. When stimulation occurred with other ammonium salts, it was due to osmosis or concentration.

**Summary: Lithium salts.**—The lithium salts examined stimulated in two ways: the chloride, iodide, nitrate, and sulphate, by osmosis; the citrate, hydrate, and oxalate in some other manner, since the latter salts stimulate in solutions too weak to dehydrate the nerve. Although the iodides do not stimulate in  $\frac{m}{3}$  solutions, they come much nearer stimulation than the chlorides, thus confirming the observations on sodium salts, where the chloride was weaker than the iodide. If lithium salts be compared with the corresponding

TABLE VI.  
AMMONIUM SALTS.

Salt.	Weakest stimulating solution.	Remarks.
$\text{NH}_4\text{Cl}$	$\frac{m}{2}$ (?)	Tetanus. Limit not accurately determined.
$\text{NH}_4\text{I}$	$\frac{m}{5}$	Tetanus.
$\text{NH}_4\text{NO}_3$	$< m$	Limit not determined.
$(\text{NH}_4)_2\text{SO}_4$	$\frac{m}{3}$	Tetanus.
$(\text{NH}_4)_2\text{C}_2\text{O}_4$	$< \frac{m}{2}$	Limit not determined. Probably much lower.
$\text{NH}_4\text{OH}$	..	No stimulation at any strength.
$(\text{NH}_4)_2\text{HPO}_4$	$\frac{m}{3}$ (?)	Tetanus.
$(\text{NH}_4)_3$ citrate	$\frac{m}{3.6}$	Very long tetanus.

sodium salts, it will be found that the former occupy a position just below the latter as stimulating salts. The citrate, for example, is less powerful than sodium citrate, but more powerful than ammonium citrate, and this, in turn, is more powerful than potassium citrate. The order of stimulating action is the same in the salts of other corresponding acids; the oxalate of sodium being a stronger stimulant than that of lithium, which is stronger than ammonium oxalate, and the last stronger than potassium.

**Rubidium salts.**—Rubidium chloride was the only rubidium salt examined. This salt is far more powerful as a stimulant than sodium chloride. In its effect on the nerve it strongly resembles sodium

hydrate. Its weakest effective stimulating solution was an  $\frac{m}{20}$ , thus placing it close to the hydrate in stimulating power. It is easily the most powerful stimulating chloride I have found. The latent period is very short: one to three minutes; the contractions are tetanic and quickly over. The irritability of the nerve, in  $\frac{m}{8}$  solution, is lost very rapidly. In Experiment 1313 the nerve was non-irritable throughout to the strongest induction shocks, after twenty-five minutes immersion. The irritability of the nerve was restored by transferring it to sodium chloride  $\frac{m}{8}$ .

The stimulating action of rubidium chloride was a surprise, since in the literature it is generally stated that rubidium acts like potassium.

TABLE VII.  
LITHIUM SALTS.

Salt.	Weakest stimulating solution.	Remarks.
LiCl	$\frac{1}{2}$	Long latent. Weak stimulant.
LiI	$\frac{1}{4}$	Stronger than chloride.
LiOH	$\frac{m}{18}$	Short, high tetanus.
Li <sub>2</sub> SO <sub>4</sub>	$\frac{1}{4}$	
Li <sub>2</sub> C <sub>2</sub> O <sub>4</sub>	$\frac{m}{10}$	Long tetanus.
Li <sub>2</sub> CO <sub>3</sub>	$\frac{1}{4}$	
Li <sub>3</sub> citrate	$\frac{m}{80}$	Strong tetanus. Lasts 1 hour in $\frac{m}{20}$ solution.

Grützner<sup>1</sup> says that cæsium is a more intense stimulus than rubidium. Zoethout<sup>2</sup> remarks that when nerves are immersed in rubidium chloride nothing happens. I do not see the reason for this discrepancy. I carefully recrystallized my rubidium chloride without changing its properties. In the Bunsen flame, it gave the typical rubidium spectrum and appeared to be pure.

**Cæsium chloride.**—I anticipated that cæsium chloride would be the most intense stimulant of the nerve. My results show that this is not the case. In no case did the  $\frac{m}{8}$  solution stimulate, although it preserved irritability longer than any other chloride except the

<sup>1</sup> GRÜTZNER: *Loc. cit.*

<sup>2</sup> ZOETHOUT: This journal, 1904, x, p. 213.

sodium. This result is at variance with that obtained by Grützner, who states that caesium chloride was the most intense stimulant of the nerve. Although I recrystallized the caesium salt once, using only the portion that crystallized in the middle of the time of recrystallization, and although spectrum analysis in the Bunsen flame indicated no impurities, it may have been that the salt was impure, although I do not think this was the case. In any case, the salt was not far removed from a stimulating salt.

**Barium chloride.** — In a preliminary paper I overlooked the fact that barium salts would stimulate the nerve even in weak solutions. Mr. O. H. Brown of this laboratory kindly called my attention to the error. Barium chloride will generally stimulate in an  $\frac{1}{12}$  and probably more dilute solutions. It will also stimulate in stronger solutions. There is no doubt, hence, that this salt stimulates as sodium and some other salts do in some way other than by osmosis. The latent period is shorter than with sodium chloride solutions of the same osmotic strength. For an  $\frac{1}{10}$  solution it is about forty minutes, while for sodium chloride it is over two hours. If added to sodium chloride solutions in small amounts, barium chloride increases the stimulating action of the sodium chloride. An equivalent of barium chloride appears, therefore, to be stronger as a stimulant than an equivalent of sodium chloride. The character of the contractions differs, as a rule, from those obtained in sodium chloride. The contractions consist of very high single contractions repeated at intervals. Tetanus may, however, occur. The effect of barium chloride on the irritability of the nerve will be taken up in a later instalment of the paper. It preserves irritability for some time. I have demonstrated that the salt was acting on the nerve and not on the muscle. Besides the etherization of the nerve between the solution and the muscle, already described under sodium chloride, I have tried immersing the nerve in sodium chloride between the barium chloride and the muscle. If barium chloride should be diffusing down the nerve, or creeping along the nerve, it would in this way be so diluted as not to reach the muscle in sufficient amounts to stimulate it. Putting sodium chloride here, however, did not alter the result. From these experiments there is no doubt that the salt is stimulating the nerve, and not the muscle.

If the end of the nerve is cut off after immersion in barium chloride, the same phenomena are observed as after the same operation when the nerve has been in sodium chloride. The muscle goes into a pro-

longed tetanus, which in some cases may last nearly an hour. The tetanus is higher and more prolonged than after immersion in a sodium salt. The explanation of this tetanus is discussed in a later paper. The same salts inhibit the action of the barium chloride as the sodium, as will be shown in a later section.

The identity of the reactions in the two salts leaves no doubt in my mind that both stimulate the nerve in the same manner. We thus have further evidence that the stimulation of the nerve by chemicals is not a specific function of sodium ions. As an example of the effect of cutting off the end of the nerve after immersion, the following experiment may be quoted.

*Experiment 992.* — The nerve was immersed in  $\frac{m}{10}$  barium chloride solution. After thirty-two minutes I stimulated the end electrically with a single induction shock. But a single contraction resulted. With repeated induction shocks, tetanus was obtained which lasted only during the stimulation. One minute later, at 10.50, I cut off about two millimetres of the end of the nerve. An extreme tetanus followed, lasting nearly two minutes. At 10.52 a second portion was cut off. This was followed by a tetanus like the preceding. At 10.54 I cut the nerve off close to the muscle. A high tetanus followed, lasting ten seconds.

Barium nitrate and acetate also stimulated. The phenomena were the same as with the chloride, except that the nitrate appeared somewhat weaker. Barium hydrate stimulates even more powerfully than sodium hydrate. It stimulates in solutions stronger than  $\frac{m}{1}$ . It produces a high, short tetanus after a short latent period. Barium permanganate in two experiments stimulated powerfully in  $\frac{m}{10}$  solution. The stimulation lasted about one minute, and the nerve was quickly killed.

**Summary: Barium salts.** — All barium salts stimulated in solutions too weak to draw water from the nerve. All phenomena were identical with the phenomena produced by sodium salts except that they were more intense. This identity convinces me that barium and sodium salts stimulate in the same manner.

**Summary.** — Of the salts tested in Table VIII, only strontium hydrate stimulated in solutions isotonic or hypotonic with the nerve. The rest require a high concentration for stimulation, and all depress irritability with greater or less speed.

**Acids: Conclusions.** — From the results with acids shown in Table IX, stimulation cannot be attributed to the hydrogen ions. Since

TABLE VIII.

OTHER SALTS.

Salt.	Weakest stimulating concentration.	Remarks.	Salt.	Weakest stimulating concentration.	Remarks.
MgCl <sub>2</sub>	$\frac{m}{5}$	Tetanus in stronger.	ZnSO <sub>4</sub>	$\frac{m}{2}$	Weak stimulant.
Mg(NO <sub>3</sub> ) <sub>2</sub>	$\frac{m}{5}$	One or two contractions.	ZnCl <sub>2</sub>	$\frac{m}{4}$	Tetanus. *
MgSO <sub>4</sub>	$\frac{m}{2}$	Prolonged tetanus.	MnCl <sub>2</sub>	$>\frac{m}{8}$	None in $\frac{m}{8}$ or weaker; stronger not tried.
BeSO <sub>4</sub>	$>\frac{m}{2}$	No contractions. Stronger solutions not tried.	CoCl <sub>2</sub>	$>\frac{m}{8}$	None in $\frac{m}{8}$ or weaker; stronger not tried.
CaCl <sub>2</sub>	$\frac{m}{4}$	Tetanus.	NiCl <sub>2</sub>	$>\frac{m}{8}$	None in $\frac{m}{8}$ or weaker; stronger not tried.
Ca(NO <sub>3</sub> ) <sub>2</sub>	$<m$	Tetanus.	Pbacetate	$>\frac{m}{8}$	None in $\frac{m}{8}$ or weaker; stronger not tried.
Ca(OH) <sub>2</sub>	Concentrated.	No stimulation.	CuCl <sub>2</sub>	$>\frac{m}{8}$	None in $\frac{m}{8}$ or weaker; stronger not tried.
SrCl <sub>2</sub>	$\frac{m}{5}$	High tetanus.	CuSO <sub>4</sub>	$\frac{m}{2}$	Tetanus.
Sr(NO <sub>3</sub> ) <sub>2</sub>	$<m$	High tetanus.	AlCl <sub>3</sub>	$>\frac{m}{8}$	None. Stronger not tried.
SrBr <sub>2</sub>	$\frac{m}{3}$	Weaker than chloride.	HgCl <sub>2</sub>	Concentrated.	No contractions.
SrI <sub>2</sub>	$<\frac{m}{2}$	Tetanus.	AgNO <sub>3</sub>	$>\frac{m}{8}$	None in $\frac{m}{8}$ or weaker; stronger not tried.
Sr(OH) <sub>2</sub>	$\frac{m}{40}$	Short, high tetanus.	FeCl <sub>3</sub>	$>\frac{m}{8}$	None in $\frac{m}{8}$ or weaker; stronger not tried.

acids from the start depress irritability very rapidly, this ion is shown to be a powerful depressant. At first glance the stimulation in  $\frac{n}{7}$  HCl might lead to the inference that the hydrogen ion in itself had some stimulating powers, but the intense depression in the acids in dilute solution, and the fact *illustrated farther on, that the addition of small amounts of acid to the stimulating solutions of other salts always prevents the stimulating action of the latter is sufficient* to show that the ion itself is in reality depressant.

It is not impossible that the stimulating action of acids in concentrations greater than  $\frac{n}{7}$ , may be due to the electrical disturbance in the nerve. If the nerve tip is immersed in acid the demarcation current of the nerve is quickly abolished, but may be restored by alkalis. When the nerve is placed in acid, a concentration chain is established between the outside and inside of the nerve, the latter region being alkaline. Assuming that the interior of the nerve is neutral, a difference of about 0.5 of a volt will exist on account of

the difference of concentration of the hydrogen ions when the nerve is in  $\frac{N}{10}$  HCl. This current is closed through the nerve and may stimulate it as the nerve's demarcation current stimulates. In the muscle this action is very apparent. When an acid or oxidizing or reducing solution is run over the outside of a gastrocnemius muscle, shortening takes place at once (the so-called "contact reaction" of

TABLE IX.

## ACIDS.

Acid.	Solutions which stimulated. In terms of a N solution.	Concentrations failing to stimulate.	Weakest stimulating concentration.	Latent in minutes in weakest stimulating solution.	Character of contractions.
HCl	$\frac{3}{5}, \frac{1}{10}, \frac{1}{4}, \frac{1}{5}, \frac{1}{7}$	$\frac{6}{5}, \frac{1}{8}, \frac{1}{4}, \frac{1}{5}, \frac{1}{10},$ $\frac{1}{15}, \frac{1}{20},$	$\frac{2}{7}(?)$	1	Tetanic.
HNO <sub>3</sub>	$\frac{1}{5}, \frac{1}{2}, \frac{2}{5}, \frac{1}{3}, \frac{1}{4}$	$\frac{8}{5}, \frac{1}{10}, \frac{1}{12}, \frac{1}{14}$	$\frac{N}{7}(?)$	1	Short, high tetanus.
H <sub>2</sub> SO <sub>4</sub>	2, 1, $\frac{2}{3}, \frac{3}{4}, \frac{7}{8}, \frac{1}{2}, \frac{1}{5},$ $\frac{1}{10}$ (once)	1, $\frac{3}{4}, \frac{1}{5}, \frac{1}{10}, \frac{1}{20},$ $\frac{1}{40}$	$\frac{N}{3}$	1½	Short tetanus.
Lactic	$\frac{1}{2}, \frac{1}{3}, 1, \frac{1}{4}$	concentrated	$\frac{N}{3}$	..	Short tetanus.
Acetic	$\frac{7}{9}, \frac{7}{10}, \frac{11}{10}$	$\frac{11}{10}, \frac{1}{20}, \frac{1}{18}, \frac{1}{8}$	$\frac{N}{18}$	1	Short tetanus.
Oxalic	$\frac{1}{3}, \frac{1}{5}, \frac{1}{4}, 2, \frac{1}{2}, \frac{1}{6},$ $\frac{1}{10}, \frac{1}{18}, \frac{2}{10}$	$\frac{1}{4}, \frac{1}{6}, \frac{1}{10}, \frac{1}{18}, \frac{1}{10}$	$\frac{N}{10}(?)$	14	Tetanus. Low.
Tartaric	$\frac{1}{10}$ (in one case)	$\frac{7}{4}, \frac{7}{8}, \frac{7}{12}, \frac{7}{20}, \frac{7}{40},$ $\frac{1}{10}, \frac{2}{10}$	....	..	A few isolated.
H <sub>3</sub> PO <sub>4</sub>	....	3, 8, 2, $\frac{2}{5}, \frac{2}{3}, \frac{3}{10},$ $\frac{1}{10}, \frac{1}{4}$	....	..	0
Citric	$\frac{1}{10}, \frac{1}{30}$ (seldom)	$\frac{1}{10}, \frac{1}{30}, \frac{1}{20}, \frac{1}{40}$	$\frac{N}{10}(?)$	2	Very low tetanus. High in strong solutions.
H <sub>2</sub> O*	....	....	....	..	Isolated contractions in some cases.

\* Dr. W. E. GARREY states that the irritability of Chilomonas is also greatly increased by distilled water. This is possibly due to the fact that in distilled water the protoplasm is brought nearer the neutral point.

Loeb and Zoethout). This contraction takes place too promptly and involves too many of the fibres of the muscle to be due to diffusion inward of the acid or salt. It appears to me that this sudden contraction is most probably due to the concentration-chain electrical effect already mentioned. This contraction is something quite different from the rhythmic contraction of muscle produced by sodium chloride.



TABLE X.

THE OSMOTIC PRESSURE OF THE WEAKEST STIMULATING SOLUTIONS.

Substance.	Weakest stimulating solution.	Osmotic pressure in atmospheres.
KCl	$\frac{1}{4} - \frac{112}{3}$	11.0 - 14.4
KBr	$\frac{112}{4} - \frac{112}{3}$	11.0 - 14.4
KI	$\frac{1}{4} - \frac{1}{3}$	11.0 - 14.4
KNO <sub>3</sub>	$\frac{112}{4} - \frac{112}{3}$	11.0 - 14.0
KClO <sub>3</sub>	$\frac{112}{4} - \frac{112}{3}$	11.0 - 14.0
KHSO <sub>4</sub>	$\frac{112}{4}$	12.0
K <sub>2</sub> SO <sub>4</sub>	$\frac{112}{5} - \frac{112}{4}$	12.6 - 12.8
CaCl <sub>2</sub>	$\frac{112}{4}$	13.0
SrCl <sub>2</sub>	$\frac{112}{5} - \frac{112}{4}$	11.4 - 14.0
MgCl <sub>2</sub>	$\frac{1}{5} - \frac{1}{4}$	11.2 - 13.7
Mg(NO <sub>3</sub> ) <sub>2</sub>	$\frac{112}{5} - \frac{112}{4}$	11.0 - 13.4
MgSO <sub>4</sub>	$\frac{112}{2}$	15.3
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	$\frac{112}{3}$	16.37
NH <sub>4</sub> Cl	$\frac{112}{2} (?)$	19.0 (?)
LiCl	$\frac{112}{2} - 3\frac{112}{5}$	16.5 - 19.6
LiI	$\frac{112}{4} - \frac{112}{3}$	11.0 - 14.0
(NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub>	$\frac{112}{3}$	13.0 (?)
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	$\frac{112}{3}$	16.4
K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	$\frac{112}{3}$	15.8
ZnSO <sub>4</sub>	$\frac{112}{2} - m\frac{1}{5}$	15.2 - 17.2

### III. DISCUSSION OF RESULTS.

The foregoing results show that electrolytes stimulate in two ways: first by their concentration (osmosis), and second, in some other way.

**1. Stimulation by concentration.** — An inspection of Table X shows that nearly all compounds, irrespective of their nature, stimulated in concentrations of from 11-14 atmospheres osmotic pressure. This is the case even for salts which by themselves are strong depressants,

such as calcium chloride. This pressure is the same as that required for stimulation by solutions of non-electrolytes, and we may conclude that the stimulation is not due to the electrical condition of the electrolyte. The fact that the non-electrolytes stimulate in about the same concentration as the electrolytes, indicates that the stimulation is due to the extraction of water producing a certain change in the nerve-substance, and not because the extraction of water increases the concentration of the electrolytes in the exterior of the nerve, thus causing a concentration-chain action. Were the latter the case, the electrolytes should stimulate in more dilute solutions than the non-electrolytes. There are certain variations in the limits of stimulating concentrations for different salts. Lithium salts, and particularly the chloride, require a slightly higher osmotic pressure in order to stimulate than almost any other salts. On the other hand, acids will stimulate in solutions a little less concentrated than most other salts. Potassium salts again stimulate, when they do stimulate, very quickly and forcibly. Magnesium and zinc sulphates require a somewhat higher osmotic pressure in their solutions than fourteen atmospheres to stimulate. The cause of these variations is undoubtedly, I think, in part owing to the varying speeds of diffusion of the different salts. Acids, which diffuse most quickly owing to the great velocity of the hydrogen ion, stimulate in strong solutions very quickly; lithium salts diffuse very slowly, owing to the slow rate of movement of the lithium ion; the sulphates of magnesium and zinc also diffuse slowly, and these compounds require a slightly higher initial concentration to stimulate. Where they move slowly, the dehydration takes place less abruptly.

2. **The other cause of stimulation.** — In an isotonic solution of sodium chloride there are four elements to which the stimulation might be due. These are the sodium atom, the chlorine atom, the positive charge of electricity attached to the sodium atom, and the negative charge of electricity borne by the chlorine atom.

Since the majority of the sodium salts stimulate, the first suggestion is that this stimulation is a specific and peculiar property of the sodium ions.

This hypothesis cannot be upheld, for nearly all barium and rubidium salts, and some potassium, ammonium, lithium, and strontium salts also stimulate in the same circumstances. Furthermore not all sodium salts stimulate. The difference between the stimulating power of these different salts is only one of degree. All

rubidium, nearly all barium, the majority of sodium, many lithium, a few ammonium, a few potassium salts, and an occasional strontium salt will stimulate. It is clear that there is nothing peculiar and specific in the stimulating action of sodium salts.

The chemical composition of the anion may also be disregarded as unimportant. Nearly all salts of sodium stimulate, whatever the anion is. Since such salts as potassium hydrate and sodium chloride, which have nothing in common from a chemical point of view, produce the same effect, it is fair to assume that their chemical composition is not the important factor in their action. Nevertheless they must have something in common, since they produce the same result. They are alike in this particular: *They are both electrolytes.*

We are hence led at once to examine their electrical condition, to determine the cause of their action. Each solution contains positive and negative particles. Since the chemical composition of these ions is unimportant, we come by exclusion to their electrical condition. Their action must be due to this.

*It is clear that if the effect is produced by the electrical charges, chemical stimulation is essentially electrical and the laws of electrical stimulation can be applied to chemical stimulation.* The first and most fundamental of these laws is that stimulation at the make of the current occurs at the cathode or negative electrode, and depression occurs at the positive electrode or anode. From these laws, it is clear on this hypothesis that the anion or the chlorine in sodium chloride must be the stimulating agent owing to its negative charge and that the positive sodium must have a depressing action owing to its positive charge. I believe that the facts may be explained on this hypothesis and strongly indicate its truth.

We may now proceed to examine the facts from the point of view that the anion stimulates while the cation depresses, and that their action is due to the electrical charges.

#### A. THE ACTION OF ALKALIES AND ACIDS.

In solutions of the alkalies the very powerful hydroxyl ion is predominant. The facts show that the stimulating action of solutions of the alkalies is to be referred to this ion, since the stimulating power of all hydrates is about the same, and the limit in each is about a  $\frac{n}{20}$  solution. In a solution of any hydrate, therefore, we have the conditions which our theory demands. The hydroxyl ion is a nega-

tively charged ion; the properties of all alkaline solutions are due to these ions; and in this case the negative ion is certainly preponderant, whatever the explanation of that predominance is. *The fact that the hydrates are powerful stimulants, bears out, therefore, the hypothesis that the negative ion is the stimulating ion.*

In acids there is the opposite condition of a powerful, positive ion, the hydrogen ion. The activity of this ion is so great that it predominates in mineral acids and overbalances the action of the negative ion. If we consider the action of acids on the nerve, it is found that, without exception, the mineral acids in isotonic solutions which ionize most completely, annihilate irritability, or depress the nerve very strongly. In other words, those acids which show the effects of the hydrogen ions in the most typical manner, depress without stimulation, and depress powerfully. *This clearly shows that this positive ion has a depressant action, and implies that the sodium ion which resembles the hydrogen ion in carrying a positive charge, also depresses, but for some reason not so strongly.* The only exceptions to the statement that the acids depress without stimulation are oxalic acid and possibly citric, which, in certain concentrations, have a weak stimulating tendency. In these acids, however, we may be dealing in part with the effect of the anion, for the oxalates are always among the most powerful stimulants. *The action of both alkalies and acids bears out the hypothesis that the positive ions depress and the negative stimulate.*

#### B. THE RELATION OF THE VALENCE OF THE ANION OR CATION TO ITS STIMULATING OR DEPRESSING POWER.

If the action of the anion is due primarily to the electrical charges it has upon it, the more charges present the greater should be the stimulating power. That a relationship might exist between the valence of an ion and its physiological action could be inferred from the work of Schulze,<sup>1</sup> Linder and Picton,<sup>2</sup> and Hardy,<sup>3</sup> who have shown that such a relationship exists between the power of salts to precipitate colloids and the valence of their ions.<sup>4</sup> Inasmuch as

<sup>1</sup> SCHULZE: Journal für praktische Chemie, 1882, xxv, p. 431; 1883, xxvi, p. 320.

<sup>2</sup> LINDER and PICTON: Chemical journal, 1895, lxvii, p. 63.

<sup>3</sup> HARDY: Proceedings of the Royal Society, 1900, lxvi, p. 110.

<sup>4</sup> For a bibliography of the colloids, see MÜLLER: Zeitschrift für anorganische Chemie, 1904, xxxix, p. 121.

colloids are present in protoplasm, and as it has been suggested by Graham<sup>1</sup> and many observers that protoplasmic activities are dependent, in part, at least, upon alterations in the state of the colloids, it was a reasonable hypothesis that such a relation between ion valence and physiological action would be found to exist.

This relation was first suggested by Loeb.<sup>2</sup> Loeb thought that his results, obtained upon fundulus eggs, showed that there existed a toxic and an antitoxic action between single, double, and triple-charged cations. He stated that the poisonous action of pure sodium chloride or potassium chloride could be neutralized by any doubly-charged cation salt, such as barium, magnesium, or calcium, and by still smaller quantities of the trivalent aluminium. Loeb stated that the antitoxic action must be due to the valence of the cation, since all bivalent cations acted equally strongly, and the trivalent cations acted still more strongly. My own results on fundulus show that this statement of Loeb's is not entirely correct. There is a great difference in the power of calcium, magnesium, barium, and strontium, to neutralize the poisonous action of sodium chloride. A still greater difference exists for lead or nickel salts. Loeb's facts, therefore, do not show that antitoxic action is due to valence rather than to chemical composition.

a. **The importance of the valence of the cation.** — From the preceding tables it is apparent that no salt of a bivalent metal (cation), with the exception of strontium hydrate and barium salts, will stimulate in solutions isotonic with the nerve. On the contrary, all depress activity, and some of them with great rapidity, as, for example, mercury. Of the trivalent metals, aluminium and ferric chloride, the latter destroys irritability very fast; while aluminium chloride does not destroy irritability so rapidly as iron; it does not stimulate, but depresses. On the other hand, of the monovalent salts of sodium, ammonium, potassium, lithium, and rubidium, some of each stimulate. From these facts we may draw the conclusion that the chances of a salt containing a bivalent or a trivalent cation stimulating are greatly less than salts which contain a monovalent cation. So far as there is any relation between valence and the action of the cation, we may therefore say that *the stimulating power of any salt*

<sup>1</sup> GRAHAM: "The colloid may be looked upon as the probable primary source of the force appearing in the phenomena of vitality." *Philosophical Transactions*, 1861, cli, p. 183.

<sup>2</sup> LOEB: *Archiv für die gesammte Physiologie*, 1901, lxxxviii, p. 68.

*diminishes with an increase in the valence of the cation, or the depressant action of the salt increases with an increase in the number of charges on the cation.* This conclusion plainly bears out the hypothesis that the cation is depressant, and its depressant action is due to the positive charges it bears. The rule has, however, several marked exceptions, since monovalent silver salts depress strongly, as do acids, while bivalent barium salts stimulate. The cause of these exceptions will be given under the heading of solution tension, on page 481.

There is, however, no sign of the quantitative relationships observed by Hardy, and apparently found by Loeb, between ionic valence and the physiological or precipitating power of ions. Loeb's observations are in part incorrect, and in such a way as to make the resemblance between his results and Hardy's less striking than formerly appeared. In my results, a strontium ion is only a little more powerful as a depressant than two monovalent sodium ions. As far as can be seen from the results so far given, the relation between the physiological action of monovalent, bivalent, and trivalent cations is more nearly a one, two, three ratio, which indicates that a proper comparison is really one of equivalents. An equivalent of calcium is more powerfully depressing than an equivalent of sodium. How much more powerful will be indicated farther on, but certainly not a hundred times more powerful.

**b. The importance of the valence of the anion.** — When we examine the anions, the relationships appear much clearer and more convincing (Table IV, p. 462). With few exceptions, sodium salts of the bivalent anions stimulate more powerfully in equimolecular or isosmotic solutions than the monovalent; and the trivalent anion salts stimulate more powerfully than the bivalent. For example, sodium chloride will stimulate only in concentrations stronger than  $\frac{m}{12}$ ; sodium sulphate will stimulate in concentrations as dilute as  $\frac{m}{30}$ , and sodium citrate in concentrations of  $\frac{m}{50}$ . The main exceptions to the rule are the hydrates, which stimulate more powerfully than a single equivalent of a bivalent anion; but this is possibly due, in part, to the presence of bivalent oxygen ions in small amounts in the hydrate solution.

The relationship in stimulating power between monovalent, bivalent, and trivalent anion salts of sodium, judging from the weakest dilution in which they will stimulate, is about as 1 : 2.5 : 4. *These facts show very clearly that, as the number of negative charges on the anion increases, the stimulating power of the salt increases.* It ap-

pears, also, that of various bivalent anions, not all of them stimulate alike, since the latent period and height of the tetanus produced vary with the different salts. While the limit of dilution which will stimulate is the same in the thiosulphate and the sulphite, the latter has a much shorter latent period and the contractions are more powerful than in the former. The cause of this variation is the ionic potential or solution tension discussed later.

c. **The action of polyvalent anions is not due to calcium precipitation or hydrolytic dissociation.** — There are two possible ways of action of the anions besides through their electrical charges which must be disproved before the action can be referred unconditionally to the charges. These polyvalent anion salts, like the citrate and oxalate, might stimulate, not by means of the many negative charges they possess, but because they precipitate the calcium in the tissues, calcium salts having an intense depressing action. This has been suggested by Loeb. Or it might be that the greater stimulating power of the citrate might be due to the hydroxyl ions present in the solutions from hydrolytic dissociation. The first possibility will be first considered.

Against the hypothesis that these salts stimulate by precipitating the depressant calcium, the following facts may be mentioned. The thiosulphate does not precipitate calcium, and yet it stimulates in as weak a dilution as the oxalate. The citrate, which precipitates calcium very slowly and imperfectly, is a more powerful stimulant than the oxalate. The monovalent fluoride which precipitates calcium, is not so strong as the sulphate in stimulating action. The formate makes an insoluble calcium salt, and yet is but slightly stronger than the chloride. The iodide, which does not precipitate calcium, is considerably stronger than the chloride. The carbonate is a weak stimulant, while it precipitates calcium strongly. It must be remembered also that the amount of calcium in the nerves is extraordinarily little, far less than can be precipitated by the sulphate or citrate, so that even if the calcium salts are formed, the dilution is so great that no appreciable change would occur either in the solubility or state of ionization of the calcium, for many of these compounds at least. These facts show, I think, that whatever may be the relation between the power of any anion to precipitate calcium and its stimulating power, the latter property is not due to the former, but both of these properties are probably due to the same fundamental peculiarity of the anion. This peculiarity, as is indicated by my results, is the solution tension of

the ion so far as its stimulating power is concerned; and it is the same factor which determines, according to Bodländer, its calcium precipitating powers. Calcium precipitation in the tissues is not, therefore, the cause of the stimulating power of these ions. There is, however, a *general* parallelism of stimulating power and the insolubility of the calcium compounds of anions.

Nor is the stimulation due to the presence of the hydroxyl ions in the solutions. This is shown by the following facts: The sulphate is neutral, and yet it stimulates more than twice as powerfully as the chloride. Variations occur in the monovalent salts in which there is no perceptible difference in the number of hydroxyl ions, as, for example, in the chlorides and iodides. The carbonate, which contains a large number of such ions, is a bad stimulant. The number of hydroxyl ions in solutions of any of these salts is also much less than the minimum number necessary to stimulate. The minimum strength of hydrate which will stimulate is between a  $\frac{n}{20}$  and a  $\frac{n}{30}$ . None of these salts contain hydroxyl ions approaching this concentration. The following figures taken from Shields<sup>1</sup> and others, show how few are the number of hydroxyl ions present. Sodium carbonate in 0.09 *n* solution contains only 0.00596 gram molecules of sodium hydrate. In other words, a  $\frac{n}{10}$  solution of sodium carbonate contains as many hydroxyls as a  $\frac{n}{1000}$  *n* solution of sodium hydrate. Borax in a  $\frac{3}{100}$  *n* solution is only 0.92 hydrolyzed, yet borax stimulates in a solution less than  $\frac{n}{11}$ . Disodium phosphate in a  $\frac{n}{20}$  solution contains only 0.00000798 gram molecules of free sodium hydrate to the litre. The final proof may be obtained by neutralizing the alkaline citrates. Ammonium citrate, for example, may be acid in reaction. Yet it stimulates powerfully, and its stimulating power increases as one makes it neutral.

The stimulating action of the sodium and lithium salts of bivalent and trivalent acids is hence not to be ascribed to the hydroxyl ions their solutions may possess.

*An examination of the relationship of the valence of the anion and the cation to the stimulating or depressing action of the salts leads to the conclusion that the cation depresses, while the anion stimulates, for with certain exceptions, which can be explained, an increase in the number of positive charges on the cation causes an increased power of depression, while an increase in valence of the anion causes an increased stimulat-*

<sup>1</sup> SHIELDS: Zeitschrift für physikalische Chemie, 1893, xii, p. 167.



*ing action of the salt.* However, as is shown on page 484, valence in itself is unimportant. It happens that polyvalent ions have, as a rule, lower solution tensions than monovalent.

### C. THE ACTION OF ELECTRODES.

Strong confirmation of the fact that the ions have different physiological actions can be obtained by causing their separation in the nerve, and thus finding out the result on the nerve at the points respectively, where cations or anions predominate. This we can do by means of electrical stimulation. When a current is passed through a nerve, a separation of the ions takes place, the positive and negative ions passing in opposite directions. If a nerve be touched by non-polarizable electrodes of sodium chloride, at the moment of contact, sodium ions pass into the nerve at the anode, and chlorine ions at the cathode. There is, hence, for a moment, a sudden increase in the number of positive ions in the anodic region, and an increase of negative ions in the cathodic region. The stimulation always begins at the point where the negative ions are entering the nerve, and depression where the positive ions are entering. This fact furnishes very strong evidence that the negative ion is the stimulating ion.<sup>1</sup>

We may, however, approach the subject in still another way. Imagine the negative electrode or cathode broken into smaller and smaller pieces, until they are as small as atoms, each atom of substance having a negative charge on it. Such an atom is an ion. There will be no change in the nature of the action of the electrode as it becomes smaller and smaller. The smallest piece must act in exactly the same way as the largest piece. *There can, hence, be no doubt that the negative ions must act like minute negative electrodes and have the same stimulating action in the ionic state that they would possess if they were fused together into one large electrode.* Furthermore, just as the particular material of which the electrode is composed is non-important, the main thing being the charge and tension of the electrode, so it is equally clear that as the electrode is broken up the charge remains the main source of action, and the material to which the charge is attached is of secondary importance, — a conclusion which is clearly shown in the facts we have considered.

Loeb<sup>2</sup> and McCallum<sup>3</sup> have explained the stimulating action of

<sup>1</sup> This interpretation has already been given by STRONG: *Loc. cit.*

<sup>2</sup> LOEB: *Archiv für die gesammte Physiologie*, 1902, xci, p. 255.

<sup>3</sup> MCCALLUM: *This journal*, 1903, x, p. 108.

barium chloride by assuming that barium stimulates. This idea is, in my opinion, erroneous. While positive ions exist which have unsaturated affinities for positive charges, and thus act like negative ions, barium is certainly not such an ion. And even if such positive ions stimulated, the stimulation would be produced not by the positive charges, but because of the fact that such ions have unsaturated affinities for positive charges, and are to that extent negative ions as well as positive. The ferrous ion, for example, is such an ion, being at the same time both a reducing and an oxidizing ion. It is both an anion and a cation. It has both positive and negative charges which it can give up. But even the ferrous ion cannot stimulate, because protoplasm has a smaller affinity for a negative charge than the ferrous ion has.

To make certain that barium would not stimulate, I made up non-polarizable electrodes of barium chloride, calomel, and mercury. When these electrodes are touched to nerve or muscle, barium ions enter the tissue at the anode, chlorine ions at the cathode. If barium stimulates, contraction should begin at the anode, as well as the cathode, at the make of the current. I have not been able to distinguish any change of the electrotonic relations when barium chloride instead of sodium chloride electrodes were used. On stimulation of the two halves of a sartorius muscle by barium chloride electrodes, the contraction invariably began at the make of the current at the cathode, and at the break at the anode. Barium, therefore, gives no more evidence of stimulating than sodium. This evidence confirms the evidence obtained from a study of the phenomena of barium chloride stimulation already given on page 468, — a study which led to the conclusion that the stimulation with barium is identical in nature with that of sodium chloride, only more intense. Barium chloride stimulates more than sodium chloride, for the reason that the depressant action of the barium is less strong than that of sodium, thus causing a greater predominance of the chlorine. Why the barium depresses less than the sodium is shown later.

I have tried similar experiments with electrodes of the chlorides of several different metals, *i. e.*, Co, Ni, Al, Mg, Mn, but have never seen any marked divergence from the ordinary course of electrical stimulation, even when the muscle had been immersed before stimulation for a short time in the solution of the salt, in order that the salt should be present in the tissue.

*In conclusion, therefore, electrical stimulation shows that the two ions*

*have an opposite physiological action regardless of their chemical nature, and that the anions stimulate the motor nerves, while the cations depress irritability.*

D. THE EFFECTS OF INDUCING POSITIVE OR NEGATIVE CHARGES  
IN THE NERVE.

Confirmatory evidence of this conclusion is obtained by the results of inducing positive and negative charges in the nerve. These experiments have been tried by V. Grandis,<sup>1</sup> who so arranged the nerve that it could be made to receive by induction a positive or a negative charge. V. Grandis, confirming old experiments by Galvani,<sup>2</sup> found that excitation of the nerve took place only when negative charges were induced in the nerve, never when positive charges. It is indifferent whether the nerve is charged by contact or induction.

E. THE RELATION BETWEEN THE PHYSIOLOGICAL ACTION OF IONS  
AND THEIR IONIC POTENTIAL, VELOCITY, AND WEIGHT.

The conclusion that the anions stimulate and the cations depress necessitates the farther conclusion that in sodium chloride, the chlorine must overbalance the sodium in its action. Why ions of the same number and kind of charges, as, for example, sodium and silver, differ greatly in the degree of their depressant action has not been explained.

In this division of the paper these points will be considered, and an explanation given of the cause of the predominance of chlorine in sodium chloride, and of the cause of the great predominance in activity of silver over sodium.

a. **Ionic potential or solution tension.**—The stimulating or depressing power of an electrode varies directly with its potential and current density. The same factors determine the actions of ions, or,

<sup>1</sup> V. GRANDIS: Archives italiennes de Biologie, 1902, xxxviii, p. 200.

"If one so arranges the experiment that the nerve receives a positive electrical charge, it is never possible to recognize any sign of excitation in the nerve, even though the capillary electrometer in communication with the nerve shows the latter undoubtedly to be positively charged. If, on the contrary, one charges the nerve with the same quantity of negative electricity, there ensues a powerful modification, which manifests itself by a strong muscular contraction," p. 201.

<sup>2</sup> GALVANI: De viribus electricitatis animalis in motu musculari, p. 382 (cited from V. GRANDIS).

in other words: Physiological action of any ion =  $C I$ , where  $I$  is the ionic potential and  $C$  the concentration. By potential is meant the tendency of the ion or electrode to give up its charge, or to change its electrical state. This tendency is measured by the solution tension, or, in the case of the ion, by the "Haftintensität." For convenience, and to bring out the resemblance between ions and electrodes, it may be called the "ionic potential." This will be the reciprocal of the solution tension.

In Table XI, Column II expresses in volts the solution tension of various cations in normal ionic solutions. The greater the voltage necessary to remove the charge from the ion, the more stable is the ion. The ionic potential being the reciprocal of the solution tension, it may be seen that, regarded as electrodes, the ions gold, silver, and mercury have the highest potential, while potassium and rubidium have the lowest. Column III states whether the chloride of any cation, when applied to the nerve in  $\frac{n}{3}$  solution, stimulates (S), or depresses (D) without stimulation. Column IV measures the stimulating or depressing power of the chlorides as follows: For stimulating salts, those marked S, in Column II, the figures represent the number of minutes which elapse after the nerve is placed in a  $\frac{n}{10}$  solution (for  $\text{BaCl}_2$  an  $\frac{m}{10}$ ), before the muscle contracts. These figures are only approximate, but they represent the average for a number of February frogs. For depressing salts, those marked D, the figures represent the number of equivalents of the chlorides of any cation which will just neutralize the stimulating action of one equivalent of rubidium chloride, when the latter is in  $\frac{m}{9}$  concentration. The smaller the number of equivalents required, obviously the more intense is the depressant action of the salt. Column V gives the number of equivalents of any chloride necessary to neutralize the stimulating power of one equivalent of sodium chloride in a  $\frac{n}{8}$  solution. These figures have not been determined so accurately as those in Column IV, and may require in some cases some modification.

The figures in Column IV were obtained by immersing the nerves in mixtures in various proportions of rubidium chloride and other chlorides, and determining the smallest number of molecules of any other chloride which would be just sufficient to neutralize the stimulating power of one molecule of rubidium chloride in a  $\frac{n}{9}$  solution. Accurate, quantitative comparisons cannot be made, but the differences between different salts in neutralizing power are so large as to permit of general comparisons.

From an inspection of this table, it is seen *that all chlorides which stimulate have cations with a very high solution tension, or a very low ionic potential.* Furthermore, the lower the cation ionic potential, the more powerfully does the salt stimulate, *c.f.* rubidium. Judging

TABLE XI.

I. Cation.	II. Solution tension volts.	III. Chloride stimulates or depresses.	IV. Stimulating or depressing power.	V. Equivalents necessary to neutralize one equivalent of NaCl.
Rb	3.00+	S	Latent 1'-2'	No neutralization.
K	2.92	D	1.5	<0.14
Ba	2.54 (?)	S	Latent 20'	No neutralization.
Na	2.54	S	Latent 150'	No neutralization.
Sr	2.49 (?)	D	3	0.1 (2)
Li	2.39	D	2	0.5
Mg	2.26 (?) 1.48	D	..	0.1
Ca	2.28 (?) 1.88	D	1+	0.02
Al	0.999	D	2	0.3
Mn	0.798	D	..	0.04
Zn	0.493	D	..	0.016
Ni	-0.049	D	0.78	
Pb	-0.128	D	0.40	
H	-0.277	D	0.12	<0.04
Cu	-0.606	D	0.16	<0.02
Hg	-1.627	D	0.028	<0.003
Au	-1.356	D	0.006	

from the latent period, rubidium chloride is sixty to one hundred times as powerful a stimulant as sodium chloride. Barium chloride, also, stimulates somewhat more powerfully, molecule for molecule, than does sodium chloride, but if equivalents be compared, the difference between them is not great. Thus an  $\frac{m}{4}$  solution of sodium

chloride has a latent period of only twenty to thirty minutes, and often less, while barium chloride of  $\frac{m}{s}$  has about the same latent period. The reason why barium chloride stimulates much like sodium chloride is clear from this table. Both cations have about the same solution tension. It is not necessary to assume, as McCallum has done, that barium stimulates. I have shown on p. 468 that barium chloride stimulates in the same way, though not to the same degree as sodium chloride. *Since those salts stimulate of which the cation is most inert, stimulation is not due to the cation*, for were it due to this ion, stimulation should be most powerful in salts of which the cation is most unstable, and has a high ionic potential.

*Depression is a function of the cation, and of its electrical state.* This is shown by Columns IV and V. *The depressing power of the salt increases with a decrease in electrical stability of the cation.* Thus it takes two molecules of lithium chloride to neutralize the stimulating action of one molecule of rubidium chloride; but only 0.8 of an equivalent of nickel chloride is required; only 0.16 of copper chloride, and 0.006 of an equivalent of gold chloride. Column V shows that the same relationships prevail for sodium chloride, but this salt being less powerfully stimulating, far less of a depressing salt is required than to neutralize rubidium. Leaving the depressant potassium chloride to be explained, the results permit the following conclusion: *The depressant power of any cation varies inversely with its solution tension, or directly with its ionic potential, i. e., with its electrical instability.*

For the anions, Table XII expresses the relation of stimulating action of sodium salts to the electrical state of the anions. Column II gives the solution tension of a few anions, and Column III enables a comparison of stimulating powers by means of the latent period; the longer the latent period, the weaker stimulant is the salt. The solution tension of most anions is, unfortunately, so uncertain as not to permit of a more extended comparison.

With the exception of the fluoride, it will be seen that *the more stable the anion, i. e., the higher its solution tension, the less stimulant the salt.* In the iodate is an anion so inert electrically, or of so low a voltage, that sodium iodate depresses. The iodide is a stronger stimulant than the chloride and its ionic potential is higher. The oxalate and hydrate, which are certainly unstable, stimulate powerfully.

*Stimulation is hence a function of the anion, and is directly proportional to the electrical instability of the anion. This strongly confirms the hypothesis that the anions stimulate by means of their electrical condition.*

An examination of other anions also confirms this hypothesis. The sulphite, for example, is an unstable anion, readily oxidized. It gives up a negative charge easily. The sulphite stimulates powerfully.

These facts show that the physiological action of any ion is a function, in part at least, of its electrical stability. The more stable the ion (the lower its potential), the more inert it is, and *vice versa*. The facts show also, I think, beyond question, the opposite action of the two ions, and the truth of the hypothesis that, for the motor nerve at least, the cation depresses, the anion stimulates. *Chemical stimulation apart from stimulation by osmosis is thus shown to be in reality electrical in its character.*

TABLE XII.

Anions.	Solution tension in volts.	Latent period in minutes for Na salts in $\frac{g}{g}$ concentration.
IO <sub>3</sub>	2.356	Depresses.
Cl	1.694	150-240
Br	1.270	150-200
I	0.79	60-120
BrO <sub>3</sub>	0.727	30
Fl	2.28(?)	20
Oxalate	0.109	10
OH	<0.557(?)	5
Citrate	0.2	10

**b. Other factors determining ionic action.** — Exceptions to the law just stated will be noticed. Potassium chloride depresses, whereas potassium has a high solution tension; sodium fluoride stimulates, whereas the fluorine ion is supposed to have a very high ionic stability. Sodium iodide is not very greatly more powerful than the chloride as a stimulant, although iodine has a solution tension only half that of chlorine. Were the solution tension or electrical stability the sole factor determining ionic action, the iodides of lithium and strontium should stimulate; but they do not. It is, therefore, clear that while ionic stability is the main factor determining the degree of ionic action, it is not the sole factor. I accordingly turned

to the other properties of ions for an explanation of these exceptions. The velocity of different ions varies. It seemed probable that this must necessarily determine in part differences in efficiency of different ions.

The faster an ion moves the more completely will it separate itself from the opposite ion, the greater will be its mean free path, and the more efficient physiologically it should be. Physiological action should vary directly with velocity and inversely with solution tension, or  $\text{Action} = \frac{I}{E}$ . By this correction, potassium ceases to be exceptional. The high velocity of this ion, *i. e.*, 65.3, is so much greater than sodium, *i. e.*, 44.4, that the potassium is actually more depressant than the sodium, in spite of its somewhat smaller ionic potential. The enormous activity of hydrogen and hydroxyl ions is also explained, since in the former case this ionic velocity is 318 and in the latter 174. The general improvement in relationships produced by this correction is marked. Many exceptions remain unexplained, however, particularly the low efficiency of the iodide and the great efficiency of rubidium and barium salts. Also this correction would make lithium salts stimulating, since by the above formula the relative depressing powers are sodium, 17.48; lithium, 14.64; and potassium, 22.36.

*Ionic weight.*—The other main variation in ions is their weight. That this may be important is shown as follows: while ionic action is due to the charge, the atom relieved of its charge will have an opposite action. Suppose this action to be proportional to its weight. Physiological action, therefore, will vary directly with the charge, and inversely with the atom (atomic weight).

$$\text{Action} = \frac{\text{Ionic potential}}{\text{Equivalent weight}} = \frac{I}{E} = \frac{I}{E \cdot wt}$$

The introduction of this factor clears up many of the exceptions. Thus iodine, which by its potential should be a very powerful stimulant, is reduced nearly to the level of chlorine, because of the depressant action of its atom. Barium, of the same potential as sodium, becomes a weaker depressant because of its greater weight.

After several trials, the following formula was found to permit a computation of the action of many ions. This formula is tentative only. Possibly the introduction of a factor representing more ac-



curately the mean free path of the ion would give a more satisfactory expression.

$$\text{Stimulating action of the anion} = \frac{V_a^2}{E_a W_a^{\frac{1}{2}}}.$$

$$\text{Depressing action of the cation} = \frac{V_c^2}{E_c W_c^{\frac{1}{2}}}.$$

In which  $V^2$  is the square of the velocity;  $E$  is the solution tension, and  $W$  is the *equivalent weight*.

Why the action of an ion should be proportional to the square of the velocity, or why the efficiency of the atom should be proportional to the square root of the equivalent weight, I do not see. These values, however, give much closer agreement with the observations than any other powers that I have tried. It may be recalled that the velocities of the molecules of two simple gases are also proportional to the square roots of their atomic weights.

These formulæ give numerical values of the ions, as is shown in Table XIII. The data are not altogether satisfactory for solution tension in particular, but while quantitatively incomplete, they are interesting.

The values used for  $V$ , were taken from Kohlrausch and Holborn's Tables, and represent  $V$  at infinite dilution. I have used these values in computing the action, at all concentrations, for the reason that at great dilution, the other factors of ionic and molecular friction involved in the velocity numbers are reduced to a minimum, and the figures probably represent more nearly the actual relative velocity of the ions at any moment, than the figures given for greater concentrations. The values for solution tension were taken for  $\frac{1}{10}N$  solutions. The figures in the table represent, therefore, the relative depressing power of the cations in  $\frac{1}{10}$  ionic solutions; and the stimulating power of the anions in the same concentration. For those ions of which the solution tension is negative,  $E$  has been put in the numerator, since for these ions the depressing power will vary *directly* with the numerical value of the solution tension. It is impossible with this formula to compare directly the numerical values of ions having a positive sign with those having a negative sign, since, owing to the fact that the solution tension becomes zero, the depressing value appears to pass through infinity.

By an inspection of Table XIII, it is seen that the numerical values of ions of high solution tension indicate fairly well the physiological

activity of the ion. Thus the bromides are known to be more depressant than the chlorides. The table shows that the bromine ion has a stimulating value markedly less than that of chlorine or iodine. The iodides certainly stimulate more powerfully than the chlorides, and corresponding with this the iodine ion has a stimulating value

TABLE XIII.

TABLE SHOWING DEPRESSING POWER OF CATIONS AND STIMULATING POWER OF THE ANIONS AS COMPUTED FROM FORMULA ON PAGE 487.

Cations.	Depressing power.	Anions.	Stimulating power.
Ba	154.19	IO <sub>3</sub>	90.05 (computation uncertain.)
Rb	157.89	Br	377.09
Na	158.28	Cl	416.27
Cs	131.54 (?)	I	462.26
$\frac{1}{2}$ Sr	189.59	OH	6,308.5
$\frac{1}{2}$ Li	194.66	$\frac{1}{2}$ SO <sub>4</sub>	318.0 (computation uncertain.)
$\frac{1}{2}$ Mg	396.00	$\frac{1}{2}$ C <sub>2</sub> O <sub>4</sub>	4,335.9 (computation uncertain.)
$\frac{1}{2}$ Ca	312.49	C <sub>2</sub> H <sub>3</sub> O <sub>2</sub>	579.8 (computation uncertain.)
K	229.36	BrO <sub>3</sub>	659.05
$\frac{1}{3}$ Al	401.11		
$\frac{1}{2}$ Mn	553.64		
$\frac{1}{2}$ Zn	758.18		
$\frac{1}{2}$ Co	5781.5		
$\frac{1}{2}$ Ni	5496.1		
$\frac{1}{2}$ Pb	-36.66		
$\frac{1}{2}$ Cu	-250.88		
Ag	-295.17		
H	22,045.8		

(462.26) greater than chlorine (416.27). The hydrates all stimulate powerfully, and this corresponds with the very high value of the O H ion. The computation for the iodate is so uncertain as to render its numerical value almost valueless; nevertheless, the iodate is seen in any case to possess a very small stimulant power.

The bromate, on the other hand, when computed in the same manner as the iodate, is found to have a high stimulating power, and the bromates certainly stimulate the nerve powerfully. With the cations, the various ions arrange themselves very nearly in the order in which they occur in the experiments. Barium and rubidium are less depressant than sodium, and their corresponding salts accordingly stimulate more powerfully. Lithium and strontium are more depressant than sodium; potassium has a value of 229.36, showing it to be an intense depressant. The only prominent exception among the cations is cæsium, which ought to depress less than sodium, whereas cæsium salts do not stimulate so powerfully as sodium. This exception cannot at present be explained. Rubidium, also, does not have a value sufficiently low to explain the intense stimulation by rubidium salts; although a better agreement between its observed and calculated value is reached, if the speed of its diffusion is taken into account.

In spite of these few exceptions, therefore, for all simple anions and cations the results obtained by the formula place the ions in the same order, and nearly in the same numerical relation to each other as regards power, as the experimental evidence indicated.

The complex anions cannot, with present information, be computed in this manner, for the reason that the factor  $E$ , for most of them, is altogether uncertain. I hope, however, that the true solution tension of these ions, or rather their ionic potential, will soon be determined, and when that is done, their true physiological value computed. I have computed the value of the oxalate and acetate ions indirectly from the heat of formation, deducting from this the heat of formation of their decomposition products, *i. e.*, carbon dioxide and ethane. I believe from the physiological action that this value for the oxalate ion is at least approximately correct.

c. **Computation of the stimulating and depressing power of any salt.**—From the foregoing values the total stimulating power of a salt may be computed as follows:

The stimulating power varies directly with the anion, and *inversely* with the efficiency of the cation.

$\pi = \frac{A}{C}$  where  $\pi$  = the stimulating power. Substituting the values for  $A$  and  $C$  just derived.

$$\pi = \frac{A}{C} = \frac{\frac{V_a^2}{E_i W_i^{\frac{1}{2}}}}{\frac{V_c^2}{E_c W_c^{\frac{1}{2}}}} = \frac{V_a^2 E_c W_c^{\frac{1}{2}}}{V_c^2 E_i W_i^{\frac{1}{2}}}$$

In computing for any given concentration, a correction must be made for the change in solution tension from normal to this concentration. This change equals  $\frac{0.057}{n}$  volts for each ten times the dilution is increased or diminished. In place of  $E_c$ , therefore, the value  $E_c + \frac{0.057}{n} \log \frac{1}{c}$  must be substituted.  $V_a^2$  is given either by the transport numbers of Hittorf, or by the conductivity measurements.<sup>1</sup>

TABLE XIV.

STIMULATING ACTION MEASURED BY THE RATIO OF THE ANION TO THE CATION FOR  $\frac{1}{10}$  SOLUTIONS.

Stimulate.		Depress.	
NaOH	39.85	$\frac{1}{2}$ SrI <sub>2</sub>	2.438
$\frac{1}{2}$ Sr(OH) <sub>2</sub>	33.28	LiI	2.375
LiOH	32.41	$\frac{1}{2}$ SrCl <sub>2</sub>	2.196
$\frac{1}{2}$ Na <sub>2</sub> C <sub>2</sub> O <sub>4</sub>	27.39	LiCl	2.138
NaCO <sub>2</sub> CH <sub>3</sub>	3.663	KI	2.015
NaI	2.921	$\frac{1}{2}$ SrBr <sub>2</sub>	1.989
$\frac{1}{2}$ BaCl <sub>2</sub>	2.700	LiBr	1.937
NaCl	2.630	KCl	1.815
RbCl	2.637	KBr	1.644
NaBr	2.382	$\frac{1}{2}$ CaI <sub>2</sub>	1.479
		$\frac{1}{2}$ CaCl <sub>2</sub>	1.332
		$\frac{1}{2}$ CaBr <sub>2</sub>	1.207
		$\frac{1}{2}$ MgCl <sub>2</sub>	1.051
		HCl	-0.0189
		AgCl	-1.410

Inasmuch as the number of ions varies in different salts at the same concentration, it is necessary to introduce a factor to represent this variable. The expression must hence be multiplied by  $a$ , which represents the per cent of dissociation at concentration  $c$ .

Finally a correction must be made for the speed of diffusion. It is obvious from the laws of electrical stimulation, by which the stimu-

lating power is an inverse function of the time required to reach a certain intensity of current, that the same factor must prevail also here. Given two salts, one of which has a slightly greater speed of diffusion than the other, it is clear that it will thereby stimulate more powerfully. This factor is represented by the diffusion coefficient  $D$  of the salt. This is obtained by the formula: <sup>1</sup>

$$D = 0.04485 \frac{uv}{u+v} [1 + 0.0034 (t - 18)]$$

The formula thus corrected is as follows:

$$\pi = a D \frac{V_a^2 \left[ E_c + \frac{0.057}{n} \log \frac{1}{c} \right] W_c^{\frac{1}{2}}}{V_c^2 \left[ E_a + \frac{0.057}{n} \log \frac{1}{c} \right] W_a^{\frac{1}{2}}}$$

The figures given in Table XIV have been computed by the foregoing formula, leaving out  $a$  and  $D$ , and represent the stimulating power of the salt as measured by the ratio between the anion and the cation. The figures have not been corrected for  $D$ , nor for dissociation differences, but represent what the relative stimulating powers would be in  $\frac{n}{10}$  solutions, if all the salts had the same rate of diffusion and were equally dissociated.

By an inspection of this table it may be seen that to stimulate the motor nerve of the frog all salts must have a ratio equal to, or greater than the ratio of  $\frac{Br}{Na}$  or 2.382. The stimulating power of all salts is greater as their ratios are greater than this number. All salts having a ratio lower than this depress, and they depress the more powerfully the smaller the ratio. Thus calcium bromide is shown to be an intense depressant, since the ratio is only 1.207, or about one-half that necessary to stimulate.

The exact limiting ratio of stimulation will, of course, vary with the changes in irritability in the nerve, and there will be variations between different tissues. Thus the sciatic nerves of summer frogs require for stimulation salts having a ratio of about 3.0.

The ratio thus obtained represents the ratio between the sine and cosine of an angle. I have plotted some of the values thus obtained graphically in Fig. 1. The ordinates represent the values of the

<sup>1</sup> NERNST: *Theoretische Chemie*, 3d edition, 1900, p. 361.

anions; the abscissæ, the values of the cations. The line *OA* drawn through sodium bromide representing the ratio 2.382, is approximately the neutral line, and separates all salts which stimulate from those which depress. Depressant salts are plotted with a star; stimulant salts with a cross.

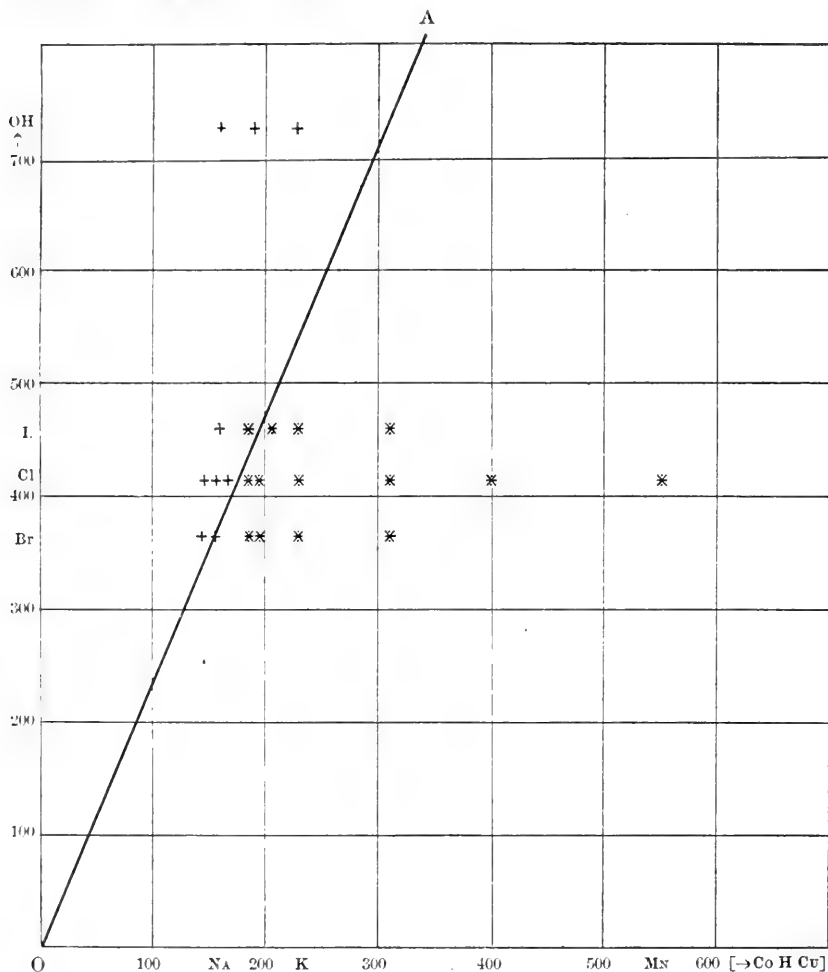


FIGURE 1.—The straight line *OA* separates the stimulating (left) from the depressing salts (right). The ordinates represent the stimulating values of the anions, and the abscissæ, the depressing values of the cations (Table XIII).

From this chart it may be seen at a glance that the hydrates of strontium, lithium, and potassium, must stimulate powerfully.

*Exceptions.* — There is one exception, *i. e.*, strontium iodide, which

lies above the stimulating line. Furthermore, rubidium chloride does not occupy the strong stimulating position it should. Lithium iodide, also, was not found to stimulate as strongly as its position would indicate. These exceptions disappear if the corrections be made for speed of diffusion and ionic concentration, or the dissociation. Any salt having a stimulating ratio will stimulate more powerfully the more rapidly it diffuses. It is clear that even though a salt have a ratio sufficient to stimulate, it may penetrate so slowly as to produce so gradual a change in the nerve as not to excite a nerve impulse strong enough to cause contraction of the muscle.

With these corrections applied the following values are obtained: for NaBr for  $D_{10}^n a_{10}^n$ , 1.963; for  $\text{SrI}_2$  for  $D_{10}^n a_{10}^n$ : 1.646. Thus strontium iodide is found to be less stimulant than sodium bromide, which agrees with the observation.

Sodium acetate has too high a value, and rubidium chloride too low. The acetate is about as powerful a stimulant as sodium chloride, while rubidium chloride is many times as powerful. When corrections are made for  $D \propto a^{1/2}$ , the following values are obtained:

NaCl	= 2.659
NaBr	= 2.409
Na acetate	= 2.428
RbCl	= 2.918

In other words, with this correction the acetate should lie in stimulating power between the chloride and bromide, which agrees with the observations. Rubidium chloride, because of its great speed of diffusion, acts as a more intense stimulant than its ratio indicates, and is thus found to be, as it is, more intense in its action than sodium chloride. Similarly, the depressant power of potassium chloride is undoubtedly enhanced by its great speed of diffusion. On the other hand, caesium chloride and sodium fluoride remain exceptions which cannot at present be explained.

In Table XV are figures which measure the stimulating power of the salt in another way. A portion of the total action of any salt is due to the anion, and will be equivalent to the anion divided by the sum of the anion and cation as follows:

$$\frac{A}{A + C} = \frac{\frac{V_a^2}{E_a W_a^{1/2}}}{\frac{V_a^2}{E_a W_a^{1/2}} + \frac{V_c^2}{E_c W_c^{1/2}}}$$

Similarly, the part of the action due to the cation is:

$$\frac{C}{A+C} = \frac{\frac{V_c^2}{E_c W_c^{\frac{1}{2}}}}{\frac{V_a^2}{E_a W_a^{\frac{1}{2}}} + \frac{V_c^2}{E_c W_c^{\frac{1}{2}}}}$$

The stimulating action of the salt must be equal to the stimulating action of the anion, plus the stimulating action of the cation. Remembering that the stimulating action of the cation is *negative*, this gives the following formula:

$$\begin{aligned} \text{Stimulating action} &= \frac{V_a^2}{E_a W_a^{\frac{1}{2}}} - \frac{V_c^2}{E_c W_c^{\frac{1}{2}}} \\ &= \frac{V_a^2 W_a^{\frac{1}{2}} - V_c^2 W_c^{\frac{1}{2}}}{E_a W_a^{\frac{1}{2}} + E_c W_c^{\frac{1}{2}}} \\ &= \frac{(V_a^2 E_c W_c^{\frac{1}{2}} - V_c^2 E_a W_a^{\frac{1}{2}})}{V_a^2 E_c W_c^{\frac{1}{2}} + V_c^2 E_a W_a^{\frac{1}{2}}} \end{aligned}$$

TABLE XV.

STIMULATING ACTION MEASURED BY THE SUM OF THE STIMULATING ACTIONS OF THE TWO IONS =  $\frac{A-C}{A+C}$ .

NaOH	0.951	LiCl	0.363
NaI	0.490	$\frac{1}{2}$ SrCl <sub>2</sub>	0.374
$\frac{1}{2}$ BaCl <sub>2</sub>	0.459	LiBr	0.319
NaCl	0.449	KI	0.337
NaBr	0.409	KCl	0.289
LiI	0.407	KBr	0.244

The results may be obtained directly from the values given in Table XIII as follows:

$$\text{For NaCl stimulation} = \frac{A-C}{A+C} = \frac{416.27 - 158.28}{416.27 + 158.28}.$$

Table XV shows that a salt must have a stimulating value of 0.409 at least in order to stimulate. In aluminium chloride, the two ions have almost the same value, and  $\frac{A-C}{A+C}$  will be nearly zero.



SUMMARY AND CONCLUSION.

The facts of chemical stimulation stated in the foregoing pages may be summarized as follows :

1. Nearly all electrolytes and non-electrolytes will stimulate the sciatic nerve of the frog in solutions having an osmotic pressure of about fourteen atmospheres. This stimulation is not due to the electrical condition of the solution, since it is common to electrolytes as well as to non-electrolytes. It is not due wholly to the extraction of water increasing the concentration of salts in the outside of the nerve and setting up thereby a concentration chain, since the non-electrolytes stimulate nearly or quite as powerfully in such solutions as the electrolytes. This stimulation is probably due to the extraction of water setting up a definite change in the colloids of the nerve, rendering the protoplasmic hydrosol unstable.

2. Many electrolytes stimulate in solutions which are so dilute as not to extract water. This stimulation is due to the electrical condition of the solution. All anions have a stimulating action; all cations, a depressing action. This is shown by the facts adduced, as well as by the well known phenomena of electrical stimulation, in which the opposite electrodes have opposite actions.

3. The ions are minute, freely moving electrodes of different voltages.

4. The physiological action of any ion depends (1) on its concentration; (2) on the sign of its electrical charge; (3) on its electrical stability or ionic potential. Its action is also modified by its velocity and weight; the faster it moves, the more powerful it is; the heavier it is, the less powerful.

5. *Physiological action is hence dependent upon the electrical state and stability of the ion and is independent of chemical composition, except as the chemical composition may influence its velocity and weight.*

6. Whether any salt stimulates or depresses, depends upon the relative efficiency of its anion and cation. If the anion markedly predominates, as in hydrates, the salt stimulates; if the cation predominates, the salt depresses.

7. Numerical values for the different ions in normal concentrations may be computed from the following formula :

$$\text{Action} = \frac{V^2}{EW^{\frac{1}{2}}}$$

$V$  = velocity;  $E$  = solution tension at normal concentrations;  $W$  = equivalent weight.

8. The stimulating power of a salt may be represented as the ratio of the anion to the cation as follows:

$$\frac{V_a^2 \left[ E_c + \frac{0.057}{n} \log \frac{1}{c} \right] W_c^{\frac{1}{2}}}{V_c^2 \left( E_a + \frac{0.057}{n} \log \frac{1}{c} \right) W_a^{\frac{1}{2}}}$$

9. To this formula corrections must be made for speed of diffusion and dissociation. Certain exceptions, *i.e.*, caesium and fluorine, were noted but not explained.

10. *Chemical stimulation is thus shown to be electrical and dependent upon the electrical charges of the ions.* Electrical stimulation is produced by modifying the distribution of ions in the nerve and thus altering their concentration as Nernst suggested.

11. Further evidence in support of these conclusions will be given in subsequent papers.

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